POSTER ABSTRACT BOOK

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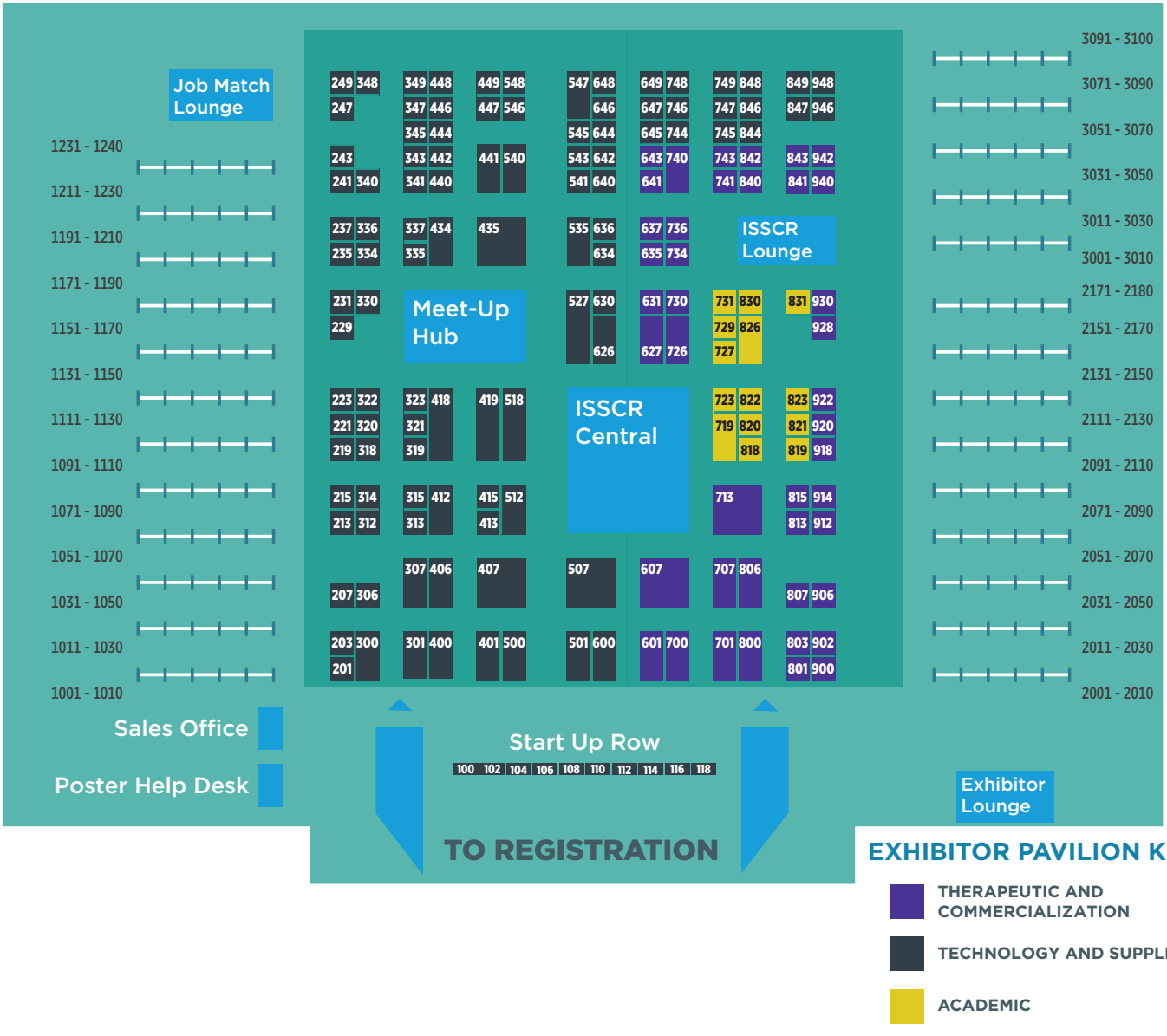

EXHIBITOR PAVILION KEY

- THERAPEUTIC AND COMMERCIALIZATION
- TECHNOLOGY AND SUPPLIERS
- ACADEMIC

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WEDNESDAY, JUNE 14, 2017

POSTER SESSION I-ODD
18:30 – 19:30

PLACENTA AND UMBILICAL CORD DERIVED CELLS

W-1001

CONCENTRATION OF THE CDCP1 PROTEIN IN HUMAN CORD PLASMA MAY SERVE AS A PREDICTOR OF HEMATOPOIETIC STEM CELL CONTENT

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Successful hematopoietic stem cell (HSC) transplantation rests upon reliable methods for HSC enumeration in sources such as cord blood (CB). Frequently used methods are the colony forming unit (CFU) assay and enumeration of CD34+ cells. However, these methods are costly and time consuming and exhaust the limited number of cells needed for transplantation. Surplus plasma from CB may contain factors that can predict or influence HSC content in CB. The aim of the study was to screen for possible biomarkers in cord plasma that correlate with the number of CFU and CD34+ cell content. Frozen, surplus cord plasma from 95 CBU was analyzed. Birth weight, gestation age, gender, mode of delivery and data on collection volume, total nucleated cell count, CD34+ cells and CFU assay were available. Cord plasma was selected based on CD34+ cell concentration and divided in two groups. Units with CD34+ cells > 50 cells/µl were considered as "high" in HSC content (n=47) and < 40 cells/µl as "low" (n=48). Samples were analyzed with Proximity Ligation Assay (Proseek Multiplex Inflammation, Olink Proteomics, Uppsala, Sweden) covering 92 protein biomarkers. There were 73 proteins (79%) detected in all samples. Two-group t-test with p-values adjusted for false detection rate (FDR) identified 5 proteins that significantly differed between the two groups. CDCP1 was the most significant (FDR adjusted p-value 0.006). Correlation with CDCP1 concentration was then investigated and was most significant between CDCP1 and CD34+ concentration (Spearman, r=0.54, 2-tailed p-value < 0.0001), but also for CFU count (r=0.30, p value 0.003) and total nucleated cell count (r=0.30, p-value 0.0004). There was no correlation with birth weight, gestation age or collection volume (Spearman), gender or mode of delivery (Mann-Whitney). In conclusion, we have identified CDCP1 as a potential biomarker of HSC content in CB. CDCP1 is a transmembrane glycoprotein regulated through tyrosine phosphorylation and its function is linked to cell adhesion. Overexpression of CDCP1 is seen in many carcinomas where it correlates with poor prognosis. This makes the identification of CDCP1 intriguing and warrants further investigation, both as a biomarker for HSC content and for its possible role in regulating the HSC presence in cord blood.

Funding Source: This work was supported by grants from The Swedish Cancer Foundation, The Swedish National Cord Blood Bank and the Sahlgrenska University Hospital.

W-1003

CELL-FREE MSC THERAPY FOR TARGETED ERADICATION OF MCF-7 USING MSC EXOSOME-DERIVED FROM 3D-PHYSIOLOGICAL OXYGEN (3%) CULTURED HWJMSCS

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Therapeutic use of MSCs-derived exosomes is a safer cell-free approach. Increasing evidence point to exosomes as paracrine mediators of the beneficial effects on tissue remodeling associated with cell therapy.To facilitate the translation of the preclinical studies of MSCs-exosomes to patients, standardization of MSC production with a focus on in vitro culture methods and precise cell characterization becomes paramount. Cell-secreted vesicles such as exosomes are non-viable in nature, and their proven safety profile makes them a potential candidate for cell-free therapeutic applications. In this study, we have assessed the effect of thymidine kinase laden exosomes for targeted proliferation control of MCF-7 breast cancer cell line. Fabricated native hWJMSCs-derived decellularized ECM was devoid of detectable DNA contents while maintaining the 3D nanofibrous architecture. The presence of oriented fibronectin fibers in the ECM were confirmed by the confocal microscopy. hWJMSCs cultured on 3% oxygen maintained their MSC's features and oriented themselves in the 3D ECM. hWJMSCs were phenotypically characterized and tri-lineage differentiation of hWJMSCs was confirmed. Exosomes were isolated by differential ultracentrifugation and morphological features were confirmed by Transmission Electron Microscope. Phenotypic characteristics of exosomes were authenticated by flow cytometry using CD63, CD9 and CD81 antibodies. Western blot using CD81 further confirmed the characteristic of purified exosomes.

Upon co-culture of CD63 GFP-labeled hWJMSCs and CD9 RFP-labeled MCF-7 cells, we observed the transfer of CD63-GFP labeled exosomes from hWJMSCs into the MCF-7 cancer cells indicating the active exosomal mediated communication between the cells. Moreover, MCF-7 breast cancer cells internalized the purified CD63 GFP-tagged hWJMSC exosomes added to the culture media, which was confirmed by confocal microscopy. Upon in vitro treating the MCF-7 cancer cells with hWJMSC-derived exosomes for 48 hrs, significant cell death was observed; which was further confirmed by Annexin-V & PI staining. In this study, we have described the anti-tumorigenic property of hWJMSC-derived armed-exosomes, which can be exploited for safer cell-free therapeutic application after extensive in-vivo animal studies.

Funding Source: Department of Biotechnology, Government of India

ADIPOSE, MUSCULOSKELETAL, AND CONNECTIVE TISSUE

W-1005

THE ROLE OF CDKN1A/P21 IN THE INHIBITION OF STEM CELL-BASED TISSUE REGENERATION DURING MECHANICAL UNLOADING

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Tissue regenerative health depends on constant cellular repair and regeneration by somatic stem cell lineages, a process that is stimulated by mechanical loading. Under disuse conditions, including exposure to microgravity, tissue regeneration may be impaired, resulting in significant tissue loss. We have previously identified CDKN1a/p21 as highly up-regulated in osteoprogenitor cells during microgravity unloading possibly resulting in reduced bone formation. This finding, in combination with the role of CDKN1a/p21 as a suppressor of mammalian tissue regeneration, suggests that this gene could be responsible for suppressing stem cell-based tissue regeneration in response to disuse. We therefore hypothesized that CDKN1a/p21 regulates regenerative bone formation in response to alterations in mechanical load. We tested this hypothesis by studying the skeletal phenotype and stem cell regenerative ability of juvenile and skeletally mature female KO mice, and the response of adult mice to hindlimb unloading (HU) for 15 and 30 days. Juvenile and mature KO mice exhibited increased proliferation rates and mineralized nodule formation compared to wildtypes, indicating increased regenerative potential. Osteoprogenitor cells from juvenile KO mice also exhibited incomplete terminal differentiation indicated by maintenance of multipotency following 21 day mineralized nodule assays. In-vitro differentiation capacity correlated to a significant increase in bone volume in juvenile mice but not in adult KO mice, indicating increased bone turnover in adult mice. Furthermore, cortical bone in skeletally mature KO mice showed similar characteristics to aged bone including increased cross-sectional area and perimeter, and increased stiffness. HU of mice led to decreased proliferation of bone marrow stem cell cultures between days 9 and 15 and increased bone nodule formation/differentiation at day 21 in wildtypes but not in KO mice suggesting that CDKN1a/p21 is required for regulation of bone regeneration in response to changes in load. These results indicate a novel role for CDKN1a/p21 in load-dependent osteoprogenitor proliferation and differentiation and that deletion of CDKN1a/p21 results in an age-dependent release of osteoblast proliferation inhibition and increased bone formation and turnover.

Funding Source: Supported by NNH14ZTT001N-0062 to E. Blaber, and NNH14ZTT001N-0063 to E. Almeida.

W-1007

HYPOXIA ACTIVATES NOTCH SIGNALING TO MAINTAIN STEMNESS IN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Human adipose-derived stem cells (hADSCs) are attractive material for regenerative medicine; however, their limited lifespan in in vitro culture systems hinders their therapeutic application. Recent data demonstrate that hypoxia may be beneficial for ex vivo culture of stem cells. These cells exhibit a high level of glycolytic metabolism under hypoxic conditions. However, the physiological role of glycolytic activation and its regulatory mechanisms are still incompletely understood. Our results demonstrate that 5% O_2 dramatically increased the glycolysis rate, improved the proliferation efficiency, prevented senescence, and maintained the multipotency of hADSCs. These effects were mediated by Notch signaling, which was activated in 5% O_2 condition. Intriguingly, 5% O_2 significantly increased glucose consumption and lactate production of hADSCs, which decreased back to normoxic levels upon treatment with a γ-secretase inhibitor. We also found that Notch signaling was involved in reduction of TIGAR and SCO2 expression through p53 inactivation. In

addition, activated Notch1 enhanced nuclear p65 levels, resulting in increase in glucose metabolism through the upregulation of glycolytic factor including GLUT3 and TPI. These data suggest that the Notch-HES1 signal enhanced the glycolytic pathway through p53 and NF-κB. Furthermore, our data also revealed that activated Notch1 markedly increased the transcriptional activity of hypoxia-inducible factor 1 (HIF-1). Knockdown of HIF-1α significantly attenuated glycolysis induced by activated Notch1, indicating that the glycolysis pathway is regulated by coordination of Notch signaling and HIF. Finally, modulation of glycolysis by pharmacological inhibitors dramatically affected the proliferation of hADSCs, which support our data indicating that the metabolic switch from mitochondrial respiration to glycolysis provides a growth advantage to hADSCs. Overall, our observations provide new regulatory mechanisms for the maintenance of stemness in 5% oxygen conditions. In addition, our study sheds new light on the regulation of replicative senescnece, which might have an impact for quality control of hADSCs preparations used for therapeutic applications.

W-1009

CARTILAGE REPAIR USING HUMAN PLURIPOTENT STEM CELL-DERIVED TISSUES IN SMALL AND LARGE ANIMAL MODELS

Craft, April M.[1], Juneja, Subhash[2], Gardner, Oliver[3], Hurtig, Mark[4], Whetstone, Heather[5], Nartiss, Yulia[2], Kandel, Rita[6] and Keller, Gordon[2]
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Osteoarthritis is a condition in which the articular cartilage that lines our joints progressively deteriorates, causing pain and interfering with daily living activities. One of the challenges associated with articular cartilage repair is that this tissue forms prenatally and regeneration does not normally occur after birth. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs; collectively hPSCs) are potential novel sources of articular cartilage to repair damaged tissue because they represent an early embryonic stage of development. To test this, we established a developmental biology-based approach for the efficient and reproducible generation of articular cartilage tissues from hPSCs. The derivative tissues are rich in proteoglycans and express important proteins that function to support compressive loads and lubricate joint surfaces. Importantly, they resisted ossification and remained stable in vivo for extended periods of time when transplanted subcutaneously into mice. Prior to their use in the clinic, however, these tissues must be tested in preclinical animal models of joint trauma or disease. We thus developed and utilized a small animal model of focal cartilage defect repair in which hPSC-derived articular cartilage was implanted into osteochondral defects in the rat knee. Defects implanted with hPSC-derived tissues showed significant regions of stable proteoglycan- and type II collagen-rich cartilage tissue after 6 and 12 weeks. In many cases, engrafted human cartilage was found to be congruent and laterally integrated with the rat articular cartilage. These data indicate that hPSC-derived articular cartilage engrafts and remains stable when implanted into an orthotopic and clinically relevant site. The success in the small animal model paved the way for evaluating the potential of hPSC-derived articular cartilage to repair damaged cartilage in a large animal model, and the results of our preliminary transplantation studies in sheep, though ongoing, are encouraging.

Funding Source: Krembil Foundation, McEwen Centre for Regenerative Medicine

W-1011

WNT REGULATES MUSCLE STEM CELL DIFFERENTIATION REQUIRE BETA-CATENIN

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Canonical Wnt signaling regulates muscle stem cell/myoblast differentiation, but there have been conflicting reports about the requirement for beta-catenin in adult myogenesis. To better understand the role of beta-catenin in myogenesis we generated beta-catenin null primary adult mouse myoblasts using CRISPR. Beta-catenin null cells showed no induction of classical Wnt target genes such as Axin2 after Wnt3a treatment. Null cells had an aberrant rounded morphology and greatly impaired spontaneous- and Wnt3a-induced differentiation. The specific requirement for beta-catenin in differentiation was confirmed by rescue experiments using beta-catenin transfection. Wildtype myoblasts showed a strong reduction in Pax7 protein expression coincident with onset of differentiation after Wnt treatment, in contrast beta-catenin null cells retained Pax7. The myogenic miRNAs miR133b and miR206 were shown to posttranscriptionally repress Pax7 expression. Beta-catenin null cells failed to induce miR133b and 206 after Wnt treatment, suggesting that beta-catenin is needed to relieve Pax7-mediated inhibition of differentiation. Beta-catenin null cells showed delayed induction of key myogenic markers such as myogenin and myosin heavy chain after Wnt

treatment, and RNAseq analysis confirmed a strong delay in activation of the global myogenic program. Moreover, ChIPseq showed that Wnt3a increased MyoD binding at E-box elements in wildtype but not beta-catenin null cells. Finally, we identify the membrane fusion protein Myomaker as a novel effector of Wnt signaling in myoblasts. Myomaker was induced by Wnt3a in wildtype but not beta-catenin null cells, and analysis of the Myomaker promoter suggests that it is activated by Wnt via MRFs such as MyoD. In summary, we propose that Wnt/beta-catenin signaling regulates muscle stem cells differentiation by: 1.Inducing miRNA mediated-Pax7 degradation to relieve Pax7's inhibitory effect on differentiation; 2.Positively regulating pro-differentiation factors including Myomaker via MRFs.

W-1013

INVOLVEMENT OF ADAM19 IN THE FATE DECISION OF MURINE CARDIAC NEURAL CREST CELLS

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The neural crest is well characterized by its migratory ability and multipotency. In vertebrate development, neural crest cells migrate out from the dorsal neural tube to the body periphery and differentiate into various kinds of cell types such as neurons, melanocytes, and bone cells. Therefore, the correct differentiation of the neural crest at the right place is important for tissue development. We focused on a transmembrane molecule, A disintegrin and metalloprotease 19 (Adam19) which is highly expressed in developing cardiac neural crest. Previously, our group reported that Adam19 in neural crests is required for ventricular septation of the heart(Komatsu et al. 2006). In this meeting, we will report: a novel role of Adam19 in cardiac neural crest development; a molecular cascade responsible for the fate decision of cardiac neural crests in vivo. These results indicate that neural crest has a novel protection system to prevent abnormal differentiation.

W-1015

UNDERSTANDING EARLY HUMAN SKELETAL MYOGENESIS TO ENHANCE SKELETAL MUSCLE PROGENITOR CELL SPECIFICATION FROM HUMAN PLURIPOTENT STEM CELLS

Xi, Haibin[1], Fujiwara, Wakana[1], Gonzalez, Karen[1], Liebscher, Simone[2], Van Handel, Ben[3], Schenke-Layland, Katja[2] and Pyle, April[4]
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Skeletal muscle progenitor cells (SMPCs) derived from human pluripotent stem cells (hPSCs) are promising sources for regenerative medicine in treating muscle wasting disorders including muscular dystrophies and sarcopenia. In the past few years, major breakthroughs in generating hPSC-derived SMPCs have been achieved by mimicking the developmental cues critical for early myogenic mesoderm specification in model organisms. Nevertheless, the current protocols remain in large inefficient and result in highly heterogeneous cell populations unsuitable for clinical implementation. These drawbacks are reflective of insufficient SMPC specification in vitro, due to lack of knowledge of early human skeletal myogenesis in vivo. Using early human embryos at somitogenesis stages (week 4.5-5 of gestation; Carnegie stage (CS) 13-14), we have discovered novel pathways which can be modulated to enhance in vitro specification of hPSCs toward the fate of somite, the common ancestor of most skeletal muscles. To extend this work, we further studied the myogenic populations from week 5-7 (CS 14-18) human embryos. We found that the expression of the myogenic progenitor markers PAX3 and PAX7 are highly dynamic during this developmental period. In particular, the early developing limb buds (week 5 and 6; CS 14 and 16) contain only the PAX3+PAX7- migratory SMPCs, whereas in the more developed limbs (week 7; CS 18) they gradually transition to PAX3-PAX7+ embryonic/fetal SMPCs. Notably, the unique migratory property of the migrating SMPCs might be a desirable feature for systemic cell delivery to treat various skeletal muscle disorders. Thus, we performed immunofluorescent co-staining on human embryo sections and identified CMET as a candidate marker for the PAX3+PAX7- migratory SMPCs. We confirmed this finding by sorting week 5-6 human limb buds on CMET, and demonstrating in vitro myogenesis only occurred in the CMET positively but not negatively sorted cells. In the future, we will further develop cell purification strategies as well as unbiasedly profile the SMPCs through single cell RNA-sequencing in different stage human embryos/fetuses. Our studies

will not only shed light on early human myogenesis but also generate useful information on deriving optimal hPSC-SMPC populations for cell-based therapies.

Funding Source: NIH/NIAMS R01AR064327; Rose Hills Foundation Research Award; UCLA BSCRC, UCLA Muscular Dystrophy P30 Core Center Grant (NIH/NIAMS P30AR057230), UCLA CTSI (UL1TR000124) and UCLA Center for Duchenne Muscular Dystrophy (CDMD)

W-1017

CHARACTERIZATION OF RAT ADIPOSE-DERIVED STEM CELLS AND THEIR INDUCTION TOWARD A TENOCYTIC LINEAGE FOR REPAIR OF ACHILLES TENDON IN VIVO

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Recently, adipose-derived stem cells (ADSCs) have entered regenerative medicine and tissue engineering. Studies show promise in differentiating ADSCs toward mesodermal cell lineages to create bioactive tissue replacements. Musculoskeletal repair with ADSC adjuncts has shown improvement in tendon histology and biomechanics. No research to date has fully characterized ADSCs, the mechanism of tendon differentiation, or the effect of ADSCs on Achilles tendon repair in vivo. Our purpose was to characterize rat ADSCs, induce ADSC tenogenesis, and analyze ADSC influence on tendon repair. We hypothesized that differentiated ADSCs would yield superior tendon repair. ADSCs were harvested from Sprague-Dawley rats, isolated, grown in vitro, and characterized as stem cells with the following criteria: adherence to plastic confirmed by cell culture, spindle-shaped morphology confirmed by light-microscopy, specific cell surface antigen expression confirmed by flow cytometry, multilineage differentiation potential in culture confirmed by gene expression and phenotype analysis. ADSC tenogenesis was stimulated with 4 growth factors commonly used for tenogenic induction in 24 combination cocktails. We elucidated the optimal tenogenesis cocktail based on the analysis of cell morphology, immunostaining, and gene expression at 1, 2, and 3-week time points. Using the rat Achilles tendon injury model, hydrogel solutions of ADSCs were injected into surgically created defects. Healing was compared with unrepaired normal control to identify natural healing baseline and analyzed by histology, biomechanics, and qPCR. We characterized rat ADSCs as stem cells and achieved successful differentiation into multiple mesodermal lineages, including bone, cartilage, fat, and tendon, as evidenced by gene expression, histology, and immunostaining. The optimal tenogenesis cocktail produced increased expression of scleraxis, tenomodulin, collagen type-I and III, and tenascin C in ADSCs, confirmed by positive immunostaining of scleraxis and tenomodulin in vitro. Gene expression and histology data suggest that tendon defects injected with undifferentiated ADSCs or with tenogenically-differentiated ADSCs achieved superior tissue repair when compared to the untreated control.

W-1019

THE METABOLIC STATE OF QUIESCENT STEM CELLS CONTROLS THEIR FUNCTIONAL RESPONSE UPON ACTIVATION

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A key step in tissue repair is the activation of quiescent adult somatic stem cells into the cell cycle. Delays in activation strongly correlate with impaired healing. Many stem cell populations require several days to complete the first cell cycle following an injury to the tissue in which they are resident. We have recently shown that we can improve the kinetics of activation and enhance healing by transitioning quiescent stem cells into GAlert, a state in which they are primed to respond to injury. Here, we show that the cellular metabolism of quiescent stem cells strongly influences their response to activation stimuli. Using phenotypic and functional metabolic attributes of quiescent mouse skeletal muscle stem cells (MuSCs) and Fibro-Adipogenic progenitors (FAPs), we can predict the kinetics of the activation response. We use injury, pharmacologic, and genetic models to show that systemic regulation of mTORC1 signaling regulates mitochondrial biogenesis and respiration in quiescent stem cells and controls their reversible transition between states of high and extremely low injury responsiveness. Our data suggest that approaches to regulate the metabolism of quiescent stem cells may improve post-injury activation, a rate limiting step in tissue repair, and have therapeutic applications for healing, transplantation, and regenerative medicine.

Funding Source: This research was funded by grants from the National Institutes of Health (NIH AG041764), the American Federation for Aging Research (AFAR), and The Donald E. and Delia B. Baxter Foundation.

W-1021

MAPPING OF THE IN VITRO SECRETED PROTEIN PROFILE OF EQUINE MESENCHYMAL STEM CELLS AFTER INFLAMMATORY STIMULATION USING QUANTITATIVE MASS SPECTROMETRY ANALYSIS

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Similar to humans, the horse is a long-lived, athletic species. Osteoarthritis (OA) is one of the leading causes of lameness in horses, and cell-based therapies are seen as the next generation treatment for OA. Mesenchymal stem cells (MSCs) are already used in the veterinary clinic, and it is believed that MSCs exert their therapeutic effects through secreted trophic biomolecules, but little is known about the exact action of MSCs in articular joints and if MSCs of different origin exert the same effect. The purpose of this study was to compare the in vitro secreted protein profile (SPP) of MSCs derived from adipose tissue (AT) and bone marrow (BM) after stimulation with serum amyloid A (SAA) or interleukin 1ß (IL-1ß). Mature chondrocytes (CH) were included as a positive control. MSCs (P3) derived from equine sternal BM and subcutaneous AT, and mature CH (P0) were used. Cells were expanded to 70 % confluence in serum-enriched medium followed by a 24h wash in serum-free medium and culture with serum-free medium alone or supplemented with SAA (1 µg/ml) or IL-1ß (10 ng/ml). Medium was collected after 48h and the SPPs were compared using iTRAQ labeling and mass spectrometry analysis. A total of 651, 525, and 503 proteins with a mascot score > 100 were identified in the SPP from all cell types after no stimulation, stimulation with SAA, and stimulation with IL-1ß, respectively. The concentrations of 127 and 97 proteins were more than 2-fold higher in the SPP from CH stimulated with SAA and IL1ß compared to the BM-MSC and AT-MSC SPP, respectively. These proteins included a number of proteins involved in breakdown of extracellular matrix (ECM) and the inflammatory response e.g. MMP1, MMP3, MMP13, IL6, IRAP, CXCL1, CXCL6. In the SPP from BM-MSC and AT-MSC stimulated with IL-1ß the concentration of MMP-1, CXCL1, CXCL6, and CCL2 was more than 2-fold higher in the AT-MSC SPP compared to the BM-MSC SPP, and TIMP1 and TIMP3 was 2-fold or higher in the BM-MSC SPP than in the AT-MSC SPP. Stimulation with SAA did not affect these proteins. In conclusion, the composition of the SPP from BM-MSC, AT-MSC, and CH changed after stimulation with IL1ß and SAA. The results indicate that the concentration of selected proteins central in the inflammatory response and breakdown of ECM are more abundant in the SPP from CH and AT-MSC compared to the SPP from BM-MSC.

W-1023

FIGMENT, A SEP IN LINC00116, REGULATES HUMAN & MURINE ADIPOCYTE METABOLISM

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Recent genomic, transcriptional, and proteomic studies have revealed the existence of many yet unannotated small open reading frame (smORF) encoded polypeptides (SEPs). Several SEPs have been thoroughly characterized, performing functions in metabolism and development. These discoveries have led to speculation a large array of uncharted bioactive small proteins exists. Previous research has discovered lincRNAs that play a role in murine adipogenesis. Of these, Linc00116 contains a smORF that is highly conserved among vertebrates. We sought to determine if this smORF is translated and relevant to human adipose tissue biology and metabolism. Here we present the functional characterization of this SEP, termed Figment (FGM). Linc00116 expression increases during differentiation of human pluripotent stem cells (hPSCs) into adipocytes, and the FGM peptide is detected by mass spectrometry. The homolog of Linc00116 is also expressed in murine adipose tissue. In order to ascribe a function to FGM, we have used CRISPR-mediated genome editing to knock out FGM in hPSCs as well as in mice. Using these knockout models in conjunction with rescue experiments, we demonstrate that FGM modulates adipocyte metabolism. FGM KO increases lipid accumulation in hPSC-derived adipocytes; conversely overexpression reduces total triglyceride levels. This is caused by a reduction in lipolysis of the KO fat cells. FGM localizes to the mitochondria, while IP-mass spectrometry reveals association with ATP synthase, among many other mitochondrial proteins. Metabolic activity assays demonstrate that FGM overexpression reduces glycolysis and oxidative phosphorylation, but

increases fatty acid oxidation, in accord with our earlier results. To investigate the molecular effects of FGM perturbation, we performed intracellular metabolomics, finding a distorted mitochondrial bioenergetics profile, most notably in the TCA cycle. Lipidomics revealed an accumulation of long-chain triglycerides in the FGM KO adipocytes. The lipolysis defect is recapitulated in FGM KO mice, which display lowered blood triglyceride and free fatty acid levels. This work demonstrates that the novel characterized SEP FGM plays a role in the mitochondrial function of hPSC-derived adipocytes, as well as murine lipid metabolism.

CARDIAC TISSUE

W-1025

CHARACTERIZATION OF HIPSC-DERIVED CARDIOMYOCYTES FORMATTED INTO A PHYSIOLOGICALLY-RELEVANT SCREENING PLATFORM

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Current approaches on the study of acute cardiotoxicity have been transformed with the availability of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). However, lack of appropriate cell geometry and reproducible sarcomeric organization, together with accurate cardiac cell junction organization remain important challenges impeding a wider adoption of this elegant model. We have developed a novel high-density screening platform using hiPSC-CMs that emulates correct cardiac muscle fiber organization. Our platform leads to improved sarcomeric organization, as seen by readily identifiable, correctly patterned myofibrils along the cell body, opposed to frequently absent, undefined or disarrayed sarcomeric organization when cardiomyocytes are plated in standard cell cultureware. Plakogoblin, a key component of cardiac cell junctions, shows correct targeting to distal intercalated discs in our platform, as opposed to an unrestricted, peripheric localization to the cell membrane in standard cell cultureware. We also observe increased gene expression of ryr2, atp2a2, and pln, key components of cardiomyocyte calcium handling pathways, which are crucial for cardiac physiology. The expression levels of cardiac ion channel genes such as cacnac1c, scn5a, kcne1, kcnq1 as well as cardiac cell junction components gja1, gja5 and dsp are also increased. Altogether we describe a novel hiPSC-derived cardiomyocyte platform with greater physiological relevance that is pre-formatted to high throughput screening.

W-1027

EXOSOMES FROM DAMAGED HUMAN IPSC-DERIVED CARDIOMYOCYTES ARE CARDIO-PROTECTIVE AGAINST MYOCARDIAL INJURY

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Cardiovascular disease (CVD) is the leading cause of death in worldwide. The adult heart innately lacks the capacity to repair and regenerate the damaged myocardium from ischemic injury. Myocardial infarction (MI) is the most common heart disease of the CVD. MI is the irreversible death of heart cells secondary to prolonged lack of oxygen supplies. Despite of tremendous effort on research for a long time from all over the world, MI still among the leading causes of death with a high rate of morbidity and mortality. In recent years, rapid emergence of induced pluripotent stem cells (iPSCs) and iPSC-derived cardiomyocytes (iCMs) presents a valuable opportunity to replace the functional cells to the heart. Although the therapeutic effects of iPSC-derived cells have been investigated in many preclinical studies, the underlying mechanisms of iPSC-derived cell therapy are still unclear and limited engraftment of iCMs are well known. Transplanted cell retention and survival in ischemic myocardium area remains one of the huge challenges for clinical translation. Our previous study showed that the improve heart function after transplanted of iCMs to the mouse MI model. However, we could not observe live engrafted iCMs from the follow-up study and assumed that most of the beneficial effects are paracrine actions from transplanted cells. Recent evidence indicates that the stem cells exert their therapeutic action via paracrine mechanisms through exosomes. They contain unique cytoplasmic microRNAs (miRNAs) and proteins that function as intercellular messengers and effectors, controlling a wide spectrum of genetic regulation. Exosomes and their miRNAs have emerged as crucial regulators of cardiovascular function. However, the mechanism of endogenous repair in the heart or stimulates of cardioprotection is poorly understood. Our previous data showed that significantly improved survival rate of exosome treated injured iCM group than control group. We found that altered expression patterns of miRNAs in the exosome from hypoxia condition which may contribute to restored the injured cardiac function. Taken together, our data indicated that exosomes and their miR-106a-363 cluster released from damaged iCMs exert a protective role against oxidative

stress to prevent apoptosis of cardiac cell types under ischemic injury.

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W-1029

EFFICIENT CARDIAC REPAIR BY COTRANSPLANTATION OF MESENCHYMAL STEM CELLS WITH CARDIAC-COMMITTED CELLS

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Although mesenchymal stem cells (MSC) have been shown to be safe in preclinical studies of cardiovascular disease, multiple meta-analyses have debated whether functional improvement is significant or not. The cardiac differentiation from MSC is achievable using cardiogenic factors, however, the high cost and long culture period may limit the applications. Here, we developed a novel method to optimize the therapeutic outcome for myocardial infarction (MI). Treatment of MSC with apicidin, a histone deacetylase inhibitor, for 24 hours dramatically increased the expressions of cardiac markers such as GATA4, Nkx2.5, and cardiac troponin I (cTnI). In apicidin-treated MSC, stemness-related genes (Nanog, Sox2, Oct4), osteogenic marker Runx2, and adipogenic marker Ppar-γ were reduced. Interestingly, yes-associated protein (YAP), a potent oncogene that drives cell proliferation, was suppressed by apicidin treatment, and YAP-knockdown MSC showed marked increases of cardiac markers. Furthermore miR-130a, an inducer of endothelial differentiation, was also suppressed by both apicidin treatment or YAP knockdown in MSC. Using a series of comparison study, we found that both cardiac gene induction and angiogenesis were most prominent in the mixture of non-treated MSC and apicidin-treated MSC (Mix). In a mouse MI model, we show that application of Mix was strongly associated with cardiac differentiation of injected MSC, improved angiogenesis and improved cardiac performance. Taken together, our results suggest that apicidin-mediated suppression of YAP/miR-130a shifts MSC cell fate toward cardiac lineage and identify apicidin as a potential pharmacological target for therapeutic development.

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W-1031

LATROPHILIN-2 COMMITS CARDIAC MUSCLE DEVELOPMENT AND REGENERATES THE INFARCTED HEART

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In order to take advantage of the beneficial properties of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), differentiation protocols for turning PSCs into functional somatic cells are required. There has recently been increasing demand for PSC-derived cardiac cells for studies on cardiovascular disease and toxicology of drug metabolites. Specific surface markers that allow monitoring of cell subsets would be valuable in establishing the conditions under which PSCs differentiate into cardiac progenitor cells (CPCs) and cardiomyocytes (CMCs). To overcome current limitations and develop broadly applicable strategies for the enrichment of PSCs-derived cardiac cells, we conducted a microarray screen to identify cell-surface markers specific to CPCs and then focused on functional molecules such as G protein-coupled receptors (GPCRs). We found a new cardiac-specific cell surface marker, latrophilin 2 (LPHN2, ADGRL2), expressed specifically by CPCs and CMCs during mouse and human PSCs differentiation in vitro and exclusively in the heart during mouse embryonic development. LPHN2 knockout in mice was embryonically lethal owing to severe heart, but not vascular, defects. There was no difference of ectoderm (neuron) and endoderm (liver) development between wild-type and LPHN2 knockout embryos. LPHN2 knockout embryos exhibited a marked reduction in expression of transcription factors that are regulators of heart development, including Gata4, Nkx2.5, Tbx5, Tbx1, Isl1, Fgf8, and Mef2c, and a cardiac structural gene, cTnT. We also investigated the in vivo differentiation potential and therapeutic efficacy of PSC-derived LPHN2+ CPCs. LPHN2+ cells differentiated into CMCs and regenerated the myocardium when transplanted into the infarcted heart, unlike LPHN2- cells. Transplanted LPHN2+ cells improved left-ventricle systolic function, and reduced infarct size and fibrosis. Molecular pathway analysis using a phospho-antibody array showed that CDK5 was a key downstream molecule of LPHN2 that interacted in parallel with Src and induced P38MAPK phosphorylation, subsequently activating cardiac-related gene transcription. These findings provide a valuable tool for isolating cardiomyogenic progenitors and CMCs from PSCs and shed light on heart development and regeneration.

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W-1033

HIGH GLUCOSE CAUSES HUMAN CARDIAC PROGENITOR CELL DYSFUNCTION BY PROMOTING MITOCHONDRIAL FISSION: ROLE OF A GLUT1 BLOCKER

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Cardiovascular disease is the most common cause of death in diabetic patients. Hyperglycemia is the primary characteristic of diabetes and is associated with many complications. The role of hyperglycemia in the dysfunction of human cardiac progenitor cells that can regenerate damaged cardiac tissue has been investigated, but the exact mechanism underlying this association is not clear. Thus, we examined whether hyperglycemia could regulate mitochondrial dynamics and lead to cardiac progenitor cell dysfunction, and whether blocking glucose uptake could rescue this dysfunction. High glucose in cardiac progenitor cells results in reduced cell viability and decreased expression of cell cycle-related molecules, including CDK2 and cyclin E. A tube formation assay revealed that hyperglycemia led to a significant decrease in the tube-forming ability of cardiac progenitor cells. Fluorescent labeling of cardiac progenitor cell mitochondria revealed that hyperglycemia alters mitochondrial dynamics and increases expression of fission-related proteins, including Fis1 and Drp1. Moreover, we showed that specific blockage of GLUT1 improved cell viability, tube formation, and regulation of mitochondrial dynamics in cardiac progenitor cells. To our knowledge, this study is the first to demonstrate that high glucose leads to cardiac progenitor cell dysfunction through an increase in mitochondrial fission, and that a GLUT1 blocker can rescue cardiac progenitor cell dysfunction and downregulation of mitochondrial fission. Combined therapy with cardiac progenitor cells and a GLUT1 blocker may provide a novel strategy for cardiac progenitor cell therapy in cardiovascular disease patients with diabetes.

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W-1035

VISUAL INTEGRATION OF MULTIPLE OMICS DATA FROM HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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The development of biomolecular techniques such as microarrays, next-generation sequencing, and mass spectrometry has revolutionized biomedical research. The advances of these technologies have dramatically increased the amount of large scale omics data that can be generated. Although this is overall beneficial, it has created challenges related to the analysis and modelling of the large and complex data sets. To aid the understanding of the interactions of various molecules in these systems, there is a great need to use methods that can integrate omics data from different domains. In this study we have used the analysis tool InCroMAP (Integrated analysis of Cross-platform MicroArray and Pathway data) to visualize multiple omics data, in order to further the mechanistic understanding of doxorubicin-induced cardiotoxicity. Multiple omics datasets were generated from human pluripotent stem cell-derived cardiomyocytes exposed to doxorubicin for up to 48h, followed by a 12 days wash-out period. Cells were harvested at selected time points, and mRNA, microRNA, and protein were isolated. Subsequent analysis of the expression revealed approximately 7500 mRNA, 390 microRNA, and 199 proteins that show differential expression between treatment and control in at least on time point. Over-representation analysis of the differentially expressed markers revealed several interesting signaling pathways (from the KEGG database), such as cardiac muscle contraction, hypertrophic cardiomyopathy, and dilated cardiomyopathy. Visual integration of the multiple omics data for these pathways identified a clear effect on core cardiomyocyte functions upon and after the doxorubicin exposure. Several interesting patterns that showed a correlation between mRNA and protein expression, were identified. For example, there was a clear downregulation of mRNAs and proteins connected to the myofibrils during the acute doxorubicin exposure, followed by an upregulation during the recovery period. The present study demonstrates the utility of integrated analysis of multiple omics data for increasing the molecular insights into the cellular responses to doxorubicin. In addition, the study also shows the usefulness of human pluripotent stem cell derivatives as in vitro models for mechanistic studies of drug-induced toxicity.

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

W-1037

DIRECTING ARTERIAL-VENOUS IDENTITY OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS USING SOLUBLE AND ADHESIVE LIGANDS WITH FLUID FLOW-INDUCED SHEAR STRESS

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Human induced pluripotent stem cell-derived endothelial cells (iPSC-EC) hold great promise as a source of endothelial cells (EC) for scientific inquiry, drug testing, and clinical use in engineered tissues. A critical step towards their use is to determine effective methods to specify iPSC-EC into arterial or venous (AV) EC subtypes. To that end, we evaluated the effects of soluble and adhesive ligands and fluid flow-induced shear stress (all of which play a role in AV specification during embryonic development) on AV specification of iPSC-EC in vitro. Human iPSC-EC (iCell-EC, Cellular Dynamics International) were treated with combinations of soluble factors (VEGF-A, 8-bromo-cAMP) and maintained on protein coated surfaces (fibronectin, recombinant Dll4). Of these combinations, fibronectin with both VEGF-A and 8-bromo-cAMP was found to be most favorable for arterial specification, as demonstrated by the fold-increases in arterial-associated gene expression (Hey1: 4.89±1.28; Hes1: 1.45±0.13; Dll4: 3.91±0.56; all $p < 0.05$), and suppression of a key venous marker (Coup-TFII: 0.62±0.08, $p < 0.05$) relative to untreated controls. However, when iPSC-EC cultured under these conditions were additionally subjected to 15 dyn/cm2 steady shear stress for 48 hours, venous specification was favoured with a 3.2-fold (±0.38, $p < 0.001$) increase in Coup-TFII expression and downregulation of select arterial genes (EfnB2: 0.16±0.02; Hey1: 0.32±0.01; Hey2: 0.23±0.01; Dll4: 0.22±0.03; $p < 0.001$ for all) relative to static controls. Currently, we are examining the effect of pulsatile versus steady shear, in conjunction with the above treatment scheme, to direct differentiation towards AV phenotypes in order to broaden the potential applications of iPSC-EC for tissue engineering.

W-1039

IN VITRO MODELING OF BLOOD-BRAIN BARRIER WITH HUMAN IPS CELL-DERIVED ENDOTHELIAL CELLS, PERICYTES, NEURONS, AND ASTROCYTES VIA NOTCH SIGNALING FOR ANALYZING DRUG KINETICS

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The blood-brain barrier (BBB) is composed of four cell populations, brain endothelial cells (BECs), pericytes, neurons, and astrocytes. Its role is to precisely regulate the microenvironment of the brain through selective substance crossing. Because of drug efflux by the BBB, the delivery of therapeutic drugs into the brain to treat CNS diseases has been a major challenge. The BBB is also associated with brain disease, as its impairment correlates with neurodegenerative diseases. These reasons have spurred researchers to establish a BBB model for analyzing the dysfunction of neurovascular units and drug permeability in vitro. Here we generated an in vitro model of the BBB by differentiating human induced pluripotent stem cells (hiPSCs) into all four populations. When the hiPSC-derived four populations were co-cultured, ECs were endowed with features consistent of BECs, including a high expression of nutrient transporters (CAT3, MFSD2A) and efflux transporters (ABCA1, BCRP, PGP, MRP5), and strong barrier function based on tight junctions. Neuron-derived Dll1, which activates Notch signaling in ECs, was essential for the BEC specification. We performed in vitro BBB permeability tests and assessed 10 clinical drugs by nanoLC-MS/MS, finding good correlation with the BBB permeability reported in previous cases. This technology should be useful for research on human BBB physiology, pathology, and drug development.

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HEMATOPOIESIS/IMMUNOLOGY

W-1041

CD26/DPPIV, ITS SURFACE EXPRESSION AND ASSOCIATED ENZYMATIC ACTIVITY, ACTS AS A NOVEL FUNCTIONAL BIOMARKER OF HUMAN FETAL LVER-DERIVED MESENCHYMAL STEM CELL

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The ontogeny of haemopoiesis during fetal life depend upon a fully competent microenvironment to provide appropriate signals. Mesenchymal stem cells (MSC), is a major cellular component of hematopoietic microenvironment and initially identified in adult bone marrow, have also been described in fetal haemopoietic tissues where they accompany the migration of haemopoietic development. Fetal liver is a major hematopoietic site during ontogeny, yet the fetal liver MSCs are poorly characterized and fewer study addressed the role of CD26/DPPIV in those cells. In the present study, we have characterized the expression and function of CD26/DPPIV in MSCs derived from human fetal liver. MSCs were isolated from second trimester fetal livers (n=8, 11~21weeks), FACS analysis indicated that CD26 expression is highly variable among cell lines (from 8% ~78%). CD26- MSCs are enriched with nestin+ cells, with higher CFU forming ability (10 fold) and osteogenic and adipogentic differentiation ability, while CD26+ MSCs are lack of nesting expression, lower CFU forming ability and lack of osteogenic differentiation. When inhibit the DPPIV activity with Diprotin A or knockdown the CD26 expression through CD26-shRNA, the CD26+MSCs partially recover the lost colony formation and osteogenic differentiation function. Thus, we propose that CD26/DPP IV is a novel functional biomarker for fetal liver-derived MSC and further mechanistic study is under going and we would like to update thoseresults at the meeting.

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W-1043

HUMAN IPSC-DERIVED RENAL CELLS: A FRIEND OR FOE OF THE IMMUNE SYSTEM?

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In an era where personalized medicine is taking front stage, immunological effects of stem cell based therapies is gaining high relevance. The promise of autologous induced pluripotent stem cells (iPSCs) being hassle-free in terms of transplant acceptance has been shattered by recent research where scientists have identified new hurdles in the behaviour of iPSC lines and their progeny. The reprogramming procedure, expression of abnormal antigens and the differentiated cell type can impact the immunological outcome of iPSC-based therapies. The aim of the following study was to analyze the immunogenicity of human iPSC-derived renal cells in autologous and allogeneic recipients in vitro. Human primary urinary cells from healthy donors were used to generate iPSC lines, which were subsequently differentiated into renal progenitors (intermediate mesoderm) and further into proximal tubular cells. Analysis of the immune-phenotype of the differentiated renal cells revealed low expression of HLA class I molecules and absence of HLA class II under homeostatic conditions. However, HLA class I expression intensity is increased under pro-inflammatory conditions. HLA class II expression is only detectable on long-term cultivated iPSC-derived proximal tubular cells after interferon gamma stimulation. Co-culture experiments of iPSC-derived renal cells with allogeneic and autologous peripheral blood mononuclear cells (PBMCs), respectively, demonstrated no induction of T cell proliferation in both settings. However, allogeneic human iPSC-derived renal progenitor and proximal tubular cells elicit T cell activation and pro-inflammatory cytokine secretion, while autologous differentiated renal cells appear to be non-immunogenic. An increased number of regulatory T cells (Tregs) were detected after co-culturing PBMCs together with renal progenitor cells which could explain the limited immunogenicity. In order to identify and understand which pathways regulate the diminished immune response against allogeneic iPSC-derived proximal tubular cells, global gene expression profiles will be used. This study poses important implications in enabling a safe transplantation of autologous and maybe even allogeneic human iPS-derived cells for renal cell-replacement therapies in regenerative medicine.

W-1045

PULSE-PRESSURE MEDIATED CIRCUMFERENTIAL STRETCH STIMULATES HEMATOPOIETIC STEM CELL FORMATION

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During fetal development, heartbeat precedes blood circulation and definitive hematopoietic stem cell (HSC) formation in the aorta-gonad-mesonephros (AGM) region. However, how the function and growth of heart and blood vessels are linked to the development of blood is largely unknown. Blood flow-mediated shear stress on the endothelial lining of the AGM stimulates the endothelial emergence of HSCs. A zebrafish mutant for cadherin 5 (malbec), which has normal blood formation despite an early circulation arrest, enabled us to analyze the timing and intertwined roles of heartbeat in blood formation. Here we show that heartbeat and stretching of the blood vessel stimulate the HSC formation. We used three-dimensional (3D) digital Doppler ultrasound, microangiography, and time-lapse confocal microscopy to demonstrate that a beating heart, in cdh5-silenced zebrafish embryos, produces pulsation in blood vessels despite the lack of cardiac output and active blood circulation. Our time-lapse confocal imaging of cd41:eGFP+ HSCs emerging from flk1:mCherry+ endothelium from the cdh5-silenced embryos, followed by machine learning analysis of pulsating blood vessels, further establishing that pulse-pressure on the arterial endothelial lining of AGM regulates the endothelial emergence of HSCs. To understand the relationship between pulse pressure, biomechanical stretching of endothelial lining, and HSC formation, we recreated pulsating blood vessel-like conditions in a dish by applying cyclic strain on mouse E11.5 AGM-derived hemogenic endothelial cells. Cyclic strain-mediated Trpv4 activation in hemogenic endothelial cells both stimulated HSC development and rescued hematopoiesis in the silent heart (tnnt2)-silenced embryos, which lacked blood flow and heartbeat. Therefore, heartbeat-mediated biomechanical stretching of hemogenic endothelial cells in the AGM stimulates trpv4 channels during the endothelial-to-HSC transition. Our findings advance our fundamental understanding of the developmental cues and 3D microenvironmental mechanisms regulating HSC formation. Our model could advance the development of methods to utilize hemogenic endothelial cells as a potential source of bone marrow independent functional HSCs in the treatment of patients requiring HSC transplants.

Funding Source: National Institute of Health, Howard Hughes Medical Institute, American Society of Hematology, St. Baldrick's Foundation

W-1047

IMPACT OF AGING ON LONG-TERM HEMATOPOIESIS IN TRANSPLANTED RHESUS MACAQUES AS INTERROGATED BY GENETIC BARCODE CLONAL TRACKING

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Aging of the human hematopoietic system is associated with a number of changes in HSPC function, resulting in increased transplant-related morbidity and mortality, impaired immunity, and an increased risk of malignant transformation. As an increasing number of older patients are receiving hematopoietic stem and progenitor cells (HSPC) transplantation, the behavior of aged versus young HSPC at a clonal level should help provide insights into the properties associated with aging. We utilized an autologous rhesus macaque HSPC transplantation model in each young (3-5 years) versus aged (18 and 25 years) macaques to investigate clinically-relevant aspects of hematopoietic aging at a clonal level via our previously-described lentiviral genetic barcoding approach (Wu et al Cell Stem Cell 2014; Kolle et al Blood 2017). There were marked differences in the patterns of reconstitution and clonal lineage relationships comparing thousands of individual engrafting HSPC in young versus aged animals. Multilineage clones contributing to granulocytes (Gr), monocytes (Mo), B cells and T cells first appeared at or after 6.5months post-transplant in aged macaque, compared to emergence of multilineage clones by 3-4.5m in young animals. Long-term myelopoiesis (up to 24m) in both young and aged macaques was clonally stable and there were no striking differences in overall clone numbers. However aged macaques showed persistenet unilineage or highly-biased lymphopoiesis; clones contributing to Gr/Mo versus B or T lineages remained almost completely distinct or markedly biased for up to 10m in the aged macaques. In the T lineage of aged macaques, we saw two distinct clonal waves contributing, the first from T-restricted naïve T cells, and the second from multipotent clones, respectively. In

aged macaques, CD4+ naïve T cells showed high GFP%, low H2AX+, and delayed reappearance, whereas CD4+ effector memory T cells showed rapid and abundant recovery with low GFP% and high H2AX+, suggesting the majority of CD4+ effector memory T recovery originated from endogenous T cells. Clonal tracking studies in aged rhesus macaques should improve our understanding of disorders of hematopoiesis in the elderly, and help improve transplantation and other therapies in this vulnerable patient population.

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W-1049

LONG NONCODING RNA FUNCTIONS DURING HSC DIFFERENTIATION AND MOUSE ACUTE MYELOID LEUKEMIA

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We understand in great detail how cell fate choices are regulated in hematopoietic stem cells (HSCs) by key transcription factors and other master regulators. Yet, a substantial fraction of the non-coding genome is transcribed in a cell type-specific manner, producing long non-coding RNAs (lncRNA). These molecules have the potential to play key regulatory roles in hematopoiesis but their functions have remained largely unexplored. We have built all the necessary tools – meticulous transcriptome analysis of annotated and de novo assembled lncRNAs as well as RNAi tools – to functionally interrogate the roles of lncRNAs during normal differentiation and malignant transformation in mouse hematopoiesis. We first assessed lncRNA function via an in vivo RNAi screen in a model of murine acute myeloid leukemia. This identified several lncRNAs essential for leukemia maintenance, and found that a number act by promoting leukemia stem cell signatures. Leukemia blasts show a myeloid differentiation phenotype when these lncRNAs are depleted, and our data indicate that this effect is mediated via effects on the c-MYC oncogene. We have also used this resource to identify lncRNAs differentially regulated during the first cell fate decisions of HSCs. Of these, we specifically focused on mouse lncRNAs that had an annotated human lncRNA in the corresponding syntenic region, which was also similarly expressed in human cord blood progenitors. Our loss-of-function studies using mouse bone marrow transplantations have identified several of these "conserved lncRNAs" as potential regulators of lineage choice and HSC self-renewal. Overall, this study highlights the importance of lncRNAs as regulators of cell fate and provides key tools for further identification and characterization of lncRNA functions.

W-1051

TOWARDS CLINICAL TRANSLATION OF SAFE AND EFFECTIVE HEMATOPOIETIC STEM CELL GENE EDITING FOR THE CORRECTION OF SCID-X1

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The scope of genetic engineering of hematopoietic stem/progenitor cells (HSPC) has broadened from gene replacement to genome editing using artificial nucleases, enabling precise modification of endogenous genes. We exploited this strategy on HSPC to insert a functional cDNA into IL2RG gene, whose mutations cause SCID-X1, thus restoring its function and physiologic expression while avoiding the risk of insertional mutagenesis. To support the rationale and explore the safety of gene correction we developed a mouse model carrying a mutated IL2RG gene in place of Il2rg. To evaluate efficacy and safety of hematopoietic reconstitution from a limited number of corrected HSPC we performed competitive transplant with WT and IL2RG-/- HSPC and found that 10% of WT cells fully reconstitute the lymphoid compartments and that administration of a conditioning regimen before HSPC infusion is required to protect from the risk of lymphoma development from the transplanted progenitors. To validate the gene correction strategy in the disease model we developed a gene editing protocol based on CRISPR/Cas9 on murine IL2RG-/- HSPC. Upon transplant, the gene corrected cells were able to generate functional B and T lymphoid lineages, showing a clear selective advantage over uncorrected cells. The corrected cells persisted long-term in the mice and generated a functional T cell response upon in vivo challenge with a pathogen, indicating that IL2RG edited cells are able to partially correct the disease phenotype. Furthermore, by

optimizing the gene editing protocol for human HSPC we could attain the threshold of IL2RG editing required for safe and effective disease rescue. By combining donor DNA delivery by AAV6 and advanced generation ZFNs we measured ~35% HDR in the bulk treated CD34+ cells and ~13% HDR upon transplant in NSG mice. Deep sequencing performed on treated CD34+ proved the high specificity of our optimized ZFNs, with no significant modification at any of the off-target sites identified by GUIDE-Seq for earlier generation ZFNs. Finally, to establish a clinical ready protocol, we scaled up the process, treating up to 25 million cells with highly qualified reagents. These studies established safety and robustness of HSPC gene editing for SCID-X1 and will be instrumental for the design of the protocol for its first clinical testing.

W-1053

P53 STABILIZATION CAUSED BY PROGRESSIVE TELOMERE SHORTENING SPECIFICALLY IMPAIRS DEFINITIVE HEMATOPOIETIC DEVELOPMENT IN DYSKERATOSIS CONGENITA

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Dyskeratosis congenita (DC) is a bone marrow failure syndrome where patients have telomeres below the first percentile in length when compared to the rest of the population. Patients with DC come to clinical attention during childhood and have a wide range of clinical manifestations, with bone marrow failure being the major cause of death. However, the progression and molecular determinants of hematopoietic failure in DC remain poorly understood, as mouse models do not fully recapitulate the human disease. In addition, patient samples are rare and cannot address the effect of telomere deficiency on the genesis of tissue failure that occurs during hematopoietic development. Here, we use the directed differentiation of human embryonic stem cells (hESCs) to understand the consequences of DC-associated mutations on the primitive and definitive hematopoietic programs. To achieve that, we created isogenic (CRISPR/Cas9-engineered) hESCs carrying disease-associated mutations in the telomerase components TERT (TERT_P704S) and DKC1 (DKC1_A353V), and also in the shelterin component TINF2 (TIN2_K280X). These telomerase mutant hESCs have reduced telomerase activity and progressive telomere shortening in culture. Interestingly, telomere shortening causes a significant expansion of primitive hematopoietic progenitors. However, while definitive hemogenic endothelium is specified, the endothelial-to-hematopoietic transition and definitive erythro-myeloid potential are significantly ablated in DC cells with short telomeres. These observations indicate that the effects of telomere shortening on hematopoiesis are not pan-hematopoietic, but instead are specific to definitive hematopoiesis. We show that the failure to specify definitive hematopoietic progenitors is caused by the accrual of DNA damage and is mediated by p53. Genetic deletion of p53 in DC cells with short telomeres restores definitive hematopoietic specification. Likewise, telomerase reactivation in DC-mutant cells efficiently restored definitive hematopoiesis specification to normal levels. Our findings elucidate a novel role of DNA damage-induced p53 signaling in hematopoietic development and demonstrate the value of in vitro hematopoietic differentiation assays to study the pathogenesis of bone marrow failure in DC patients.

W-1055

AUTOMATED IN-LINE PRE-ENRICHMENT DEVICE WITH FACS FOR HIGHLY EFFICIENT ISOLATION OF HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cells (HSCs) are progenitor cells that give rise to myeoloid and lymphoid blood cells. Harvest and transplantation of HSCs has become an established treatment for several blood disorders, malignancies, immunological diseases and genetic abnormalities. HSCs are rare and mainly found in bone marrow (~1 in 10,000), with exceptionally low frequencies (~1 in 100,000) in peripheral blood. Therefore, highly efficient HSC enrichment is required for harvest of progenitor cells and to enable downstream gene and cell therapy applications. HSCs are commonly isolated by immunomagnetic separation, but this collection procedure can be time-consuming and result in inadequate cell purity or yield. We have developed an in-line pre-enrichment device with FACS to remove debris and non-target cells, while minimizing cell loss and cell damage, eliminating centrifugation, automating sample preparation, and ensuring high purity characteristic of FACS, in a one-step workflow. For HSC isolation, the sample pre-enrichment device combines magnetic depletion of human lineage cells and acoustic removal of debris based on size, sending enriched HSCs directly to a FACS sorter for collection without further sample manipulation. Our results showed that 6.25% HSCs (CD34+CD38-) of total events were presented to FACS when acoustics was used to remove cellular debris in a bone marrow sample, compared to 0.26% when using FACS sorting only, >24 fold enrichment. Furthermore, when magnetic depletion of hematopoietic lineage cells was combined with acoustic debris removal, >90% of lineage cells were depleted and >90% of debris was

removed. Peripheral blood mononuclear cells (PBMC) could then be pre-enriched and run through FACS to sort CD34+ HSCs at a flow rate of about 12 mL/hour, an improvement in sorting speed of > 3 fold compared to using FACS sorting only, with FACS sorting efficiency of 97% to ensure the high recovery of HSCs. Similar results are obtained for apheresis samples, and HSC cell viability is preserved. Thus in-line pre-enrichment combined with FACS has demonstrated a single workflow for automated, high speed, high efficiency, and high purity isolation of viable HSCs from peripheral blood and apheresis products.

W-1057

HAEMATOPOIETIC PROGENITORS IN THE HUMAN YOLK SAC AND AGM REGION

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The yolk sac is well-recognized as the initial site of haematopoiesis during both mouse & human development while the first haematopoietic stem cells emerge later in the aorta-gonad-mesonephros (AGM) region. However, the ongoing contribution of the human yolk sac to haematopoiesis during development remains less clear. Numbers of erythroid & non-erythroid colonies in the human yolk sac and embryo proper have previously been reported. Here we describe numbers of erythroid, mixed and myeloid colonies in the yolk sac and AGM region using the more precise Carnegie Staging system for human development. The yolk sac and AGM regions were dissected from Carnegie Stage 12-17 human embryos, obtained following medical termination of pregnancy. The methylcellulose assay was used to assess haematopoietic progenitor cells (HPCs) before & after culture. Colony forming units in culture (CFU-Cs) were counted after 10—14 days. Tissues were cultured as co-aggregates with OP9 cells and explants, both at the air-liquid interface on floating membranes, adapted from a published murine culture system developed by our group. In the yolk sac, total colony numbers remained relatively constant throughout Carnegie Stages 12-17 while numbers gradually increased in the AGM region. After 7 days in co-aggregate culture with OP9 cells, the expansion of HPCs was greater in the AGM than the yolk sac. Furthermore, myeloid colonies from the AGM region were consistently larger than those from the yolk sac at corresponding Carnegie stages. In this study we report for the first time human yolk sac and AGM HPC numbers according to their Carnegie Stage and highlight important differences between these two sites of haematopoiesis during human development.

W-1059

GENERATION OF HUMAN MYELOID DENDRITIC CELLS (MDCS) FROM INDUCED PLURIPOTENT STEM CELLS FOR THE EVALUATION OF THEIR BEHAVIOR IN AUTOIMMUNE DISEASE

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Myeloid dendritic cells (mDCs) are the major antigen-presenting cell of the immune system, activating naïve T cells to initiate adaptive immunity; they play a critical role in many immune responses, from infections to cancer. mDC dysfunction, however, has been implicated in autoimmune disease pathogenesis, particularly systemic lupus erythematosus (SLE). Studies of both primary mDCs from SLE patients as well as monocyte-derived DCs from SLE patient peripheral blood samples have provided conflicting results in terms of the specific role mDCs play in the course of disease. Several groups have reported a decreased overall population of mDCs in the blood of SLE patients with a subsequent reduction in T cell proliferation ex vivo; other groups have described an increase in T cell proliferation and increase in proinflammatory cytokine production by DCs due to aberrant Toll like receptor signaling. Human mDCs are rare in peripheral blood, comprising less than 0.1% of the cell population, thus making study of them challenging. To overcome this hurdle, we have developed a technique to generate human mDCs from induced pluripotent stem (iPS) cells. Following reprogramming, iPS cells were treated with a cocktail of growth factors and cytokines to generate definitive mesoderm and subsequently mDCs, utilizing a feeder-free chemically defined media. Differentiated cells expressed the mDC markers CD11c, CD1c, CD141 and Zbtb46. The differentiated cells also produced a cytokine profile characteristic of mDCs in response to a panel of TLR agonists. This system will allow us to better evaluate the behavior of mDCs in the context of SLE by reprogramming patient-specific PBMCs. This protocol will allow us to more definitively characterize mDC dysfunction in SLE and, eventually, elucidate genetic contributions underlying this dysfunction.

PANCREAS, LIVER, KIDNEY

W-1061

THE LYSINE SPECIFIC DEMETHYLASE LSD1 REGULATES THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TOWARDS GLUCOSE SENSITIVE CELL TYPES

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The epidemic of glucose metabolism disorders such as type two diabetes (T2DM) and insulin resistance (IR) solicits the derivation of successful regenerative medicine protocols based on the use of self-engineered spare tissues. To this end, the study of the transcriptional and epigenetic mechanisms regulating the physiology of glucose responsive tissues and differentiated intermediates in vitro is a key step towards the derivation of functional cells to be used in a clinical setting. Our team focuses on the study of the role of the lysine specific demethylase LSD1 (also known as KDM1A), a FAD-dependent histone demethylase whose function is finely tuned during development and strictly dependent on cellular metabolism. We found that LSD1 is progressively induced during differentiation of human embryonic stem cells (hESs) towards glucose sensitive cell types and that elevated LSD1 levels are essential to sustain the differentiation of hESs into functional hepatocytes. Notably, our preliminary data indicate that, already at the pluripotent stage, LSD1 occupies the promoter of key transcription factors driving the differentiation into glucose sensitive cell types. Our results suggest a priming role for LSD1 in regulating glucose response during development.

W-1063

MODELING MODY3 WITH HUMAN PLURIPOTENT STEM CELLS

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Maturity onset diabetes of the young 3 (MODY3) is the most common monogenic form of diabetes and is caused by heterozygous mutations in HNF1α. Heterozygous HNF1α mice have no detectable defect in glucose homeostasis, suggesting the importance of developing a human model. We used CRISPR-Cas9 to generate HNF1α mutants (+/- and -/-) in human embryonic stem cell lines and used an in vitro differentiation system to study pancreatic beta cell differentiation and subsequent function of the derivative beta cells. Beta cell differentiation efficiency was similar between genotypes. We purified mono-hormonal beta cells by intracellular FACS of +/+, +/-, and -/- cells and performed genome wide gene expression analysis. We confirmed many of the genes found to be dysregulated in the mouse model were also impacted in the human system including HNF4α, PCSK1, and 6GCP2. In addition, we found a large number of dysregulated genes that were not found in animal models and may be targets for the discrepancy in phenotypes between human and mouse. These genes include the transcription factor PAX4 and also ATP-sensitive potassium channels (ABCC8, KCNJ11), a potassium/sodium channel (HCN1), and the metallothionein family of genes amongst others. We examined functionality of the mutant beta-like cells and found that the HNF1α null lines failed to increase insulin secretion upon high glucose stimulation and that both the +/- and -/- cells displayed an increase in basal insulin secretion. This increase in basal insulin secretion is interesting as it mimics the phenotype of transient hyperinsulinism seen in a subset of MODY3 newborns. Loss of ABCC8 or KCNJ11 are known to lead to hyperinsulinism and may be the mechanism for the increased basal insulin secretion we see in our system. Ongoing studies are aimed at re-expressing targets of HNF1α such as PAX4, ABCC8 and KCNJ11 and determining the impact on both gene expression and functionality in the derivative beta cells. Single cell RNA-seq of beta cells derived from the various mutant lines has also been performed to examine the impact of HNF1α on beta cell heterogeneity. Our studies are defining unique biology of HNF1α in a human system and may lead to both advancements in the treatment of MODY3 patients but also uncover human specific biology with the potential for treating more common forms of diabetes.

W-1065

GLOBAL TRANSCRIPTOMIC ANALYSIS OF HEPATIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS AND ADULT HUMAN LIVER TISSUE

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Hepatocytes derived from human pluripotent stem cells (hPSC-HEP) have the potential to replace presently used hepatocyte sources applied in liver disease treatment and improve models for drug discovery. Established hepatocyte differentiation protocols are effective and generate hepatocytes, which recapitulate some key features of their in vivo counterparts. However, generating mature hPSC-HEP remains a challenge. In this study, we performed transcriptome analysis at several stages during hepatic differentiation of hPSCs (i.e. definitive endoderm (DE), hepatoblasts, early hPSC-HEP, and mature hPSC-HEP) and compared with human liver tissue samples. We selected the top 2,000 differentially expressed genes in the dataset and applied K-means clustering analysis to group these genes in 9 specific clusters representing typical developmental stages of hepatic differentiation. As expected, some differences in the transcription profiles between the hPSC-HEPs and the liver tissue samples were observed. Using functional annotation, pathway- and protein interaction network analyses, we observed the grouping of differentially expressed genes in specific clusters representing typical developmental stages of hepatic differentiation. In addition, we identified hub proteins and modules that are involved in the cell cycle process at early differentiation stages. We also identified hub proteins, which differed in expression levels between hPSC-HEP and the liver tissue controls. Moreover, a module of genes was identified which were expressed at higher levels in the liver tissue samples than in the hPSC-HEP. Considering that hub proteins and modules generally are essential and have important roles in the protein- protein interactions, further investigation of these genes and their regulators may contribute to a better understanding of the differentiation process. This may suggest novel target pathways and molecules for improvement of hPSC-HEP functionality which is required in order to bring this technology to a wider use.

W-1067

NOVEL PROGENITOR CELL POPULATIONS IN THE DEVELOPING PANCREAS AS REVEALED BY SINGLE CELL RNA SEQUENCING

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The mammalian pancreas arises through a series of coordinated events, including specification, proliferation, differentiation, and maturation. While developmental biology studies have contributed considerably to our knowledge about the signaling events and transcriptional networks that occur in the pancreatic epithelium, our understanding about the non-epithelial cells is comparatively lacking. Indeed, the pancreatic epithelium does not develop in isolation, but rather in the context of a cellular compartment termed the niche, or mesenchyme. Although classical studies demonstrated that mesenchymal niche cells are required for pancreatic development, we still know little about their cellular composition or molecular makeup. We have utilized a high-throughput, droplet-based, single-cell transcriptome sequencing technique to assemble an atlas of the cellular components of the developing murine pancreas at three timepoints throughout embryonic development. Using clustering, principal component analysis, and pseudotemporal ordering analysis, we have identified novel cell populations, including novel putative progenitor populations, in both the epithelial and niche compartments. Furthermore, for functional validation we have used an ex vivo pancreatic explant system to perform cellular ablation experiments. These functional studies have revealed that a novel niche cell population is required for proper development of the organ. In summary, these studies have revealed heretofore unknown cellular heterogeneity and function within both the epithelium and mesenchymal niche of the developing pancreas. In addition to contributing to our knowledge about the basic developmental biology of this organ, these studies will also provide important clues as to which niche cells and signaling components should be recapitulated in vitro in directed differentiation experiments aimed at generating truly bona fide beta cells from human embryonic stem cells.

W-1069

ASSESSING THE DIFFERENTIATION POTENTIAL OF HUMAN PODOCYTES BY MODIFYING KIDNEY PATIENT SPECIFIC IPS CELLS IN NPHS2 LOCUS WITH CRISPR-CAS9 TECHNOLOGY

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Adult cell reprogramming to generate induced pluripotent stem cells (iPSCs) provides an unprecedented opportunity to elucidate disease mechanisms, and promote the development of replacement therapy. We have developed a differentiation protocol for functional kidney podocytes derived from iPSC (iPSC-POD). However, purification of iPSC-POD remains challenging. Additionally, there are limited studies showing the integration of iPSC-POD into kidney models in vivo. The aim of the study was to generate a iPSC reporter line targeted to the NPHS2 locus encoding the podocyte-specific gene podocin tagged with enhanced green fluorescent protein (eGFP+) using clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9. These podocineGFP modified human iPSCs (podocineGFP-iPSCs) will enable selective isolation of iPSC-POD for cell transplantation in vivo. To generate the podocineGFP-iPSCs, two plasmids: one containing the eGFP gene (within two podocin homology arms) and podocin-specific PX335 containing the pair of sgRNAs were designed. Cas9 activity was tested by Lipofectamine2000 using immortalised podocytes (IM) as a positive control as comparison. podocineGFP-iPSC-POD were generated by the addition of iPS podocyte medium containing the additive differentiation factors retinoic acid, BMP7 and activin. iPSC-PODs were differentiated over 20 days of culture and the expression of podocin+ cells (based on GFP expression) was confirmed based on GFP expression in control IM and iPSCs. Ten days after differentiation, iPSC-PODs were injected into newborn kidneys to assess integration efficiency. Using fluorescent microscopy, cells were detected in renal cortex of postnatal day 3 newborn mouse kidneys injected intrarenally with D10 iPSC-POD. This provides proof-of-principle for iPSC-POD integration in postnatal kidneys, ideal recipients due to the ongoing developmental environment and without the need for immunosuppression. This study has generated the first podocin eGFP-iPS reporter cell lines with initial findings confirming cell integration in postnatal kidneys established. iPSC-PODs can be potentially used as a valuable tool to develop a cellular replacement therapy or a test bed to study the mechanism of human glomerular disease.

W-1071

INVESTIGATING EPIGENETIC EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON HUMAN EMBRYONIC STEM CELLS (HESCS) DIFFERENTIATION TO PANCREATIC LINEAGE

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TCDD is an endocrine disrupting chemical postulated to possess diabetogenic effects. Recent laboratory and epidemiological studies have demonstrated the association of persistent TCDD exposure to type 2 diabetes (T2D) susceptibility. However, data on the epigenetic effect of low dose TCDD exposure on organogenesis and T2D susceptibility risks is very limited. We hypothesize that low dose TCDD exposure during embryogenesis could epigenetically influence pancreatic organogenesis leading to T2D susceptibility later in life. hESCs treated with low doses of TCDD (10 and 100 pM) were subjected to reduced representation bisulfite sequencing (RRBS) to assess global DNA methylation changes. TCDD treatment resulted in nearly 2000 unique hypomethylated genes and over 2000 unique hypermethylated genes. Using DAVID analysis, we identified hypermethylated genes related to T2D, insulin signaling pathways and glucose regulation. Confirmation of this results using bisulfite sequencing of TCDD treated hESCs showed a significant hypermethylation of the T2D associated gene, CAPN10. Furthermore, we compared our methylome data (hypomethylated genes) with data of pancreatic islets from T2D patients, and identified a number of differentially methylated genes in common. Differentiation of TCDD treated hESCs to pancreatic progenitors (PP) followed by gene expression pattern analysis using reverse transcription polymerase chain reaction (RT-PCR) showed a trend of upregulation of NKX6-2 which corresponded with its hypomethylation status in our methylome data. In conclusion, low dose TCDD treatment of hESCs resulted in a number of differentially methylated genes related to T2D pathogenesis and therefore understanding the epigenetic effects of low dose TCDD exposure on T2D risk genes will enhance T2D risk management and minimization.

W-1073

INFUSED BONE MARROW DERIVED CELLS WERE REPAIRD FIBROSIS AND HAD THE FUNCTION OF PHAGOCYTOSIS TO DAMAGED CELLS

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Recently, Autologous bone marrow cells were useful for the repair therapy in liver cirrhosis and many kind of diseases. We developed the GFP/CCl4 model which monitor the GFP-positive bone marrow cell (BMC) repopulated under liver cirrhosis mice (Hepatology). In this study, we estimated characterization and function of infused BMC in liver cirrhosis using Electron Microscopy (EM) in recipient liver. C57BL/6 mice were injected with CCl4 twice a week for 4 weeks to make the liver cirrhosis. GFP-positive BMC were infused from tail vein and sacrificed at 4 weeks after BMC infusion. The liver sample was fixed using both paraformaldehyde+glutaraldehyde and made epon section. We analyzed the characterization of the infused GFP-postive BMC using both EM and Immune EM (IEM). We analyzed the image of IEM, comparing with the character of positive cells by immunohistochemistry and double fluorescent staining(Antibody:GFP,MMP9, hepatoblastmarker-Liv2,Liv8-CD44,A-6,EpCAM,CXCR4,p62,transcription regulator-maternal of inhibitor of differentiation -Maid). We analyzed some kind of gene by Real-Time PCR(Gene: p16,p21,p62,Sirt1,6,AK4,Hmox,Ncam etc). We had two kinds of GFP positive BMCs in recipient cirrhosis liver using IEM method. One group of GFP positive BMCs was similar to hepatocyte in size(15-30um) and located around fiber. MMP9 positive cells, Liv8 positive cells, Maid positive cells, CXCR4 positive cells were same. These cells were round forms and different from stellate cell or Kupffer cell in feature and had the increase of lysosome structure in cytoplasm. These cells were located on fiber in hepatic cord and repaired fibrosis. The other group cells were small size (2-5um) and located in destructive area and A6 positive cells, Liv2 positive cells, EpCAM positive cells were same. These cells had high N/C ratio and smaller than hepatocyte. These cells migrated into damaged cell area and had the phagocytic capacity. These cells were few F4/80 positive cells and smaller than Kupffer cell in size. We detected two kind of infused BMCs. The round BMCs repaird liver fibrosis and the small BMCs worked the phagocytized damaged hepatocyte and maintenance of liver

EPITHELIAL TISSUES

W-1075

EPIGENETIC REGULATION OF INTESTINAL STEM CELL DURING EXPERIMENTAL NECROTIZING ENTEROCOLITIS

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Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency and a leading cause of death in preterm infants. The intestinal epithelium is characterized by rapid and constant renewal, which occurs under normal homeostasis and after injury. In NEC, gut damage is associated with impairment of native Lgr5+ intestinal epithelial stem cells (IESC). Enhancer of Zeste Homolog-2 (Ezh2) is a member of the Polycomb Repressive Complex, which transcriptionally represses gene expression via histone methylation and deacetylation. It has been shown that Ezh2 histone methyltransferase is important in the regulation of Lgr5 during postnatal development. The aims of this study was to (i) investigate the role of Ezh2 in regulating Lgr5 expression and (ii) to evaluate the impact of Ezh2 and Lgr5 on intestinal epithelium regeneration and inflammatory response during experimental NEC. In vitro: To explore the correlation between Ezh2 and Lgr5, Ezh2 was silenced by RNA interference in IEC-18 cells. Blocking Ezh2 demonstrated a reduction in H3K27Me3, resulting in a decrease in Lgr5, Ki67 (proliferation) and Sox9 (differentiation) expression levels. The expression of inflammatory cytokines IL6 and TNFα were increased in response to the inflammation process induced by lipopolysaccharide (LPS). However, silencing the expression of Lgr5 and Ezh2 resulted in even greater increases in the levels of IL6 and TNFα. In vivo: During experimental NEC (induced by gavage feeding of hyperosmolar formula, hypoxia and LPS during postnatal days 5-9), Ezh2 and Lgr5 expression in the ileum decreased compared to breastfed controls, leading to decrease epithelium proliferation (Ki67) and differentiation (Sox9), and decreased number of goblet cells (Muc2). In addition, inflammation cytokines IL6

and TNFα were significantly upregulated in NEC. AFS cell administration rescued the impairment in Ezh2 and Lgr5 through Wnt pathway, thereby restoring intestinal regeneration and reducing epithelial inflammation. This study proves that Ezh2 regulates Lgr5+ IESC, which plays an important role mediating intestinal epithelium regeneration and inflammatory response. AFSC administration reprograms the intestinal epithelium by modulating the Ezh2 and Lgr5+ IESC activation can be beneficial during intestine injury such as NEC.

W-1077

RESERVE INTESTINAL STEM CELLS FUNCTION INDEPENDENTLY OF WNT/ BETA-CATENIN SIGNALING AND DEMONSTRATE A UNIQUE GENE EXPRESSION PROFILE

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The highly self-renewing intestinal epithelium is maintained by populations of multipotent intestinal stem cells (ISCs). Rapidly-cycling Lgr5+ ISCs, present at the crypt base, maintain the intestine under normal conditions, while slowly-cycling, long-lived, mTert+ reserve ISCs, among others, present in the "+4" crypt position, contribute to intestinal homeostasis during recovery from catastrophic injury. Given their distinct roles, these two ISC populations are likely to be regulated by different mechanisms. One of the major pathways involved in intestinal development, homeostasis and tumorigenesis is canonical Wnt/β-catenin signaling. Prior studies have shown that activation of this pathway in Lgr5+ cells leads to their clonal expansion and subsequent adenoma formation. Whether reserve ISCs respond in a similar manner is unknown. To investigate the response of mTert+ ISCs to activation of this pathway in vivo, we assessed their capacity for clonal expansion and adenoma formation using lineage-tracing analysis. Here, we report that mTert+ reserve ISCs are resistant to the effects of stabilized canonical Wnt signaling, showing neither clonal expansion nor adenoma formation during 2 months of observation, as assessed following (1) deletion of APC or (2) stabilization of β-catenin. Moreover, treatment of enteroids with the GSK3β inhibitor, CHIR99021, revealed no change in the number of mTert+ cells, further confirming their independence from canonical Wnt signaling. Taken together, these results imply that mTert+ reserve ISCs are inherently unresponsive to manipulations of canonical Wnt signaling. To identify the mechanisms responsible for this, we performed RNA- and ATAC-sequencing on sorted mTert+ and Lgr5+ ISCs. Principal component analysis and unsupervised hierarchical clustering revealed molecularly discrete populations. Specifically, mTert+ ISCs exhibited a distinct gene expression profile, including elevated levels of Wnt inhibitory genes (e.g., Dkk1, Idax, Senp2, KlF4, NFAT5 and Wnt5a) as well as cell cycle inhibitor genes (e.g., p15, p21 and p57), consistent with their slow-cycling and Wnt-independent nature. Together, our findings reveal a unique genetic signature for mTert+ reserve ISCs, supporting their potential for differential regulation and function.

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W-1079

THE SINGLE-CELL TRANSCRIPTOMICS OF CUTANEOUS WOUND HEALING

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Cutaneous wound healing – the most important tissue repair process in the mammalian skin – involves a complex interplay of epidermal and underlying dermal cells. While the dermal response to wounding has been well studied on a molecular and functional level, the molecular changes that epidermal stem cells undergo as they contribute to wound re-epithelialization are still widely unknown. This is mainly due to difficulties in differentiating wound stem cells from stem cells that do not contribute to wound healing. Here we use single-cell RNA-sequencing to study transcriptional changes in Lgr5 and Lgr6 stem cells and their progeny as they contribute to cutaneous wound healing in mice. We show that transcriptionally distinct wound stem cells can be identified 1 day, 4 days, 7 days, 10 days and more than 1 month after wounding and that these cells can be subclustered into several groups. We furthermore prove that wound cells derived from Lgr5 and Lgr6 stem cells share many features of their wound program and thus partially converge during the wound healing process. Intriguingly, when analyzing the wound cell signatures for functional features, we found that gene modules typically associated with stem cell function play only a minor role. Instead, the wound program is primarily distinguished by upregulation of ubiquitous genes linked to basic cellular physiology such as mRNA and protein homeostasis or energy metabolism. Additionally, we show that the genetic identity of a wound stem cell is

partially determined by its niche location. Our study provides the first in-depth analysis of the epidermal stem cell response to wounding at single-cell resolution and one of the first single-cell studies of a tissue repair process in vivo. In addition, our data highlight the importance of metabolic and housekeeping gene expression in stem cell function during regeneration.

W-1081

KRÜPPEL-LIKE FACTOR 5 REGULATES REGENERATION OF INTESTINAL STEM CELLS FOLLOWING GAMMA-IRRADIATION INJURY

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Ionizing radiation (IR) is a commonly used cancer therapy that targets actively proliferating cells. LGR5+ intestinal stem cells are sensitive to IR damage, but these cells are also indispensable for regeneration following IR damage. Krüppel-like factor 5 (KLF5) is a zinc-finger transcription factor expressed in proliferating cells, including LGR5+ ISCs, of the intestinal epithelium and is involved in regulation of cell proliferation and differentiation. We observed that KLF5 is expressed in the majority of the MKI67+ proliferating cells in mice undergoing intestinal regeneration following total-body γ-irradiation (TBI) injury. We crossed Lgr5-EGFP-IRES-CreERT2 mice, RosatdTomato mice, and Klf5fl/fl mice to generate LGR5+ cell lineage-traceable transgenic mice with or without inducible-conditional knockout of Klf5 (Lgr5Ctrl and Lgr5ΔKlf5). Post-irradiation intestinal regeneration is divided into two phases: the apoptotic phase during 0 to 48 h, followed by the regenerative phase during 48 to 96 h post-TBI. During homeostasis (sham-treated), Klf5 deletion resulted in a slowing of proliferation and lineage tracing from LGR5+ cells from 0 to 96 hours following tamoxifen treatment. We observed increased expression of Reg1 and Reg3g genes with Klf5 deletion in LGR5+ cells, which may provide a compensatory mechanism for proliferation in absence of Klf5 albeit at a lower rate. In TBI-treated Lgr5Ctrl mice during the apoptotic phase, Lgr5ΔKlf5 mice showed increased number of apoptotic tdTomato+ cells at an early time point and throughout the phase compared to Lgr5Ctrl mice. This indicates that KLF5 regulates DNA damage-induced cell death by regulating the early DNA damage response. Furthermore, LGR5 lineages repopulated intestinal crypts during the regenerative phase, as indicating by enlarged crypts that are entirely comprised of tdTomato+ cells. However, deletion of Klf5 abrogated the regenerative capacity, as indicated by the significantly diminished number of tdTomato+ cells in Lgr5ΔKlf5 mice. Taken together, these data suggest that KLF5 modulates the regenerative response of the intestinal epithelium through functions beyond the role of a proliferation regulator in LGR5+ ISCs and lineages.

Funding Source: This study is supported by NIH grant DK052230 awarded to V.W.Yang.

W-1083

CLONAL ANALYSIS DURING EMBRYONIC MAMMARY GLAND DEVELOPMENT REVEALS THE EXISTENCE OF UNIPOTENT STEM CELLS THAT RETAIN LONG-TERM PLASTICITY

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Recent studies on mammary stem cell hierarchy during pubertal development suggest that the morphogenesis of mammary gland relies on unipotent stem/progenitor cells. However, these studies raise the question of whether and when lineage restriction occurs during embryonic mammary development and what signals orchestrate cell fate specification in this tissue. To address these questions, we have analysed mammary cell fate specification during embryonic and perinatal development combining in vivo clonal analysis by lineage tracing with whole mount immunofluorescence and mathematical modelling of clonal dynamics. Our results show that undifferentiated embryonic mammary precursors can become lineage restricted to a basal or luminal differentiation program surprisingly early in development, with evidence for unipotency as early as E12.5 and no statistically discernable bipotency after E15.5. Mechanistically, we used the constitutive activation of the luminal determinant Notch1 to demonstrate that intrinsic cell autonomous signals can impose a specific cell fate on embryonic mammary cells. Strikingly, we found that ectopic Notch1 activation can switch the lineage potential of fully committed adult cells, as expression of active Notch1 in basal cells is sufficient to convert all targeted cells to luminal progenitors. These functional studies have important implications for understanding cell plasticity in vivo and serve to clarify how reactivation of embryonic developmental programs in adult cells could lead to cancer.

Funding Source: This work was supported by Paris Sciences et Lettres (PSL* Research University), the French National Research Agency (ANR) grant number ANR-15-CE13-0013-01 and by Labex DEEP ANR-Number 11-LBX-0044.

W-1085

IDENTIFICATION OF MOUSE OVARY SURFACE EPITHELIAL STEM CELLS BY PROTEIN C RECEPTOR EXPRESSION

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Ovarian surface epithelium (OSE) is a layer of epithelial cells covering the ovary. The stem cells in OSE responsible for both development and the regeneration post ovulation and rupture has not yet been defined unequivocally. Protein C receptor (Procr) marks stem cells in the mammary gland and blood vascular endothelium. Here we use in situ and immunostaining to document the Procr-expressing cells in the mouse OSE. Procr is expressed at the hilum and OSE regions around growing follicles or among follicles. Using in vivo lineage tracing and in vitro culture, we identify that Procr+ OSE population as a stem/progenitor cells contributing to the development of OSE and OSE repair after rupture. Ablation of Procr-expressing cells affects OSE homeostasis and OSE repair. In general, Procr marks stem/progenitor cells of the ovary epithelia.

STEM CELL NICHES

W-1089

DOUBLE EMULSION HYDROGEL MICROSPHERES: A CONTROLLED AND POWERFUL SOLUTION FOR IMPROVING POST-TRANSPLANTATION SURVIVAL OF SURVIVAL OF STEM-CELL DERIVED SPIRAL GANGLION PROGENITORS

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Regenerative approaches to restoring damaged or deficient neuron function via the transplantation of differentiated pluripotent stem cells is promising, but not without its obstacles. A major concern is the survival of differentiated cells in the target tissue post-transplantation. For example, the injection of a single-cell suspension of fully differentiated stem cell-derived neurons into the cochlea in order to restore function after sensorineural hearing loss results in a less than 0.1% survival rate post-injection, due to the particularly inhospitable environment of the cochlea. To enhance the survivability of the neurons within the cochlea, it is necessary to provide a supportive network of tissue. We have designed a process for fabricating double-layered hydrogel microspheres for the culture of cells within a microfluidic environment, using a double emulsion process of creating spheres from the self-assembling peptide amphiphile RADA16-I. Comprised of a central gel core seeded with spiral ganglion neuron progenitors encapsulated by a second layer of gel seeded with stem cell-derived Schwann cells, the microspheres provide a supportive niche for the neurons post-transplantation. This method of cell culture will be useful not only for the purposes of regenerative therapy for hearing loss, but also for any similar studies that would benefit from the specific and orderly arrangement of differing cell types within a microfluidic culture.

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W-1091

EMERGING ROLES OF HAIR FOLLICLES' TRANSIT-AMPLIFYING CELLS IN NICHE REGULATION

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Stem cells are known to be regulated by signals from the niche. However, whether and how a regenerating tissue might modify the niche reciprocally is less understood. We have found that regenerating hair follicles modify their niche actively through their transit-amplifying population. Most of the regenerative tissues employ transit-amplifying cells (TACs) that are positioned in between stem cells and differentiated progeny. In a classical hierarchical model, stem cells undergo limited divisions to produce TACs, which then proliferate rapidly to expand the system and produce diverse differentiated cell types. Although TACs are indispensable for generating tissues, they have been viewed largely as a transit point between stem cells and downstream lineages. Our studies in the hair follicles however, have unleashed some fascinating biology and unanticipated functions of TACs. Ongoing works from my lab suggest that hair follicles' transit amplifying cells (HF-TACs, also known as the matrix) orchestrate concurrent changes of the niche, including several different populations in the dermis. Together, these changes allow the skin to accommodate and support the rapidly downgrowing hair follicles and heal wounds faster in anagen. One example is HF-TACs' function in promoting dermal adipogenesis: We discovered that Sonic Hedgehog (SHH) secreted by the HF-TACs regulate the level of PPARgamma in the dermal adipocyte precursors to

promote dermal adipogenesis. This finding provides a mechanism by which hair follicle regeneration and dermal adipogenesis can be coupled tightly. In addition, this finding also uncovers a critical role for TACs in orchestrating the generation of both their own progeny (differentiated cells of the hair) and a neighboring lineage (adipocytes) to achieve concomitant tissue production across lineages. With these findings, HF-TACs have emerged as key players in tissue regeneration through coordinating tissue production, governing stem cell behaviors, and instructing niche remodeling.

W-1093

PARACRINE EFFECT OF DERMAL PAPILLA CELLS IN SKIN FIBROBLAST ACTIVATION AND HAIR GROWTH: A POTENTIAL TOOL FOR SKIN HEALING

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Dermal papilla is a central key to morphogenesis and regeneration of hair follicles, especially due to its paracrine signaling to epidermal follicular cells. Dermal papilla cells (DPCs) not only regulate hair follicle development and growth, but also act as a reservoir of multipotent stem cells with increasing therapeutic importance including skin repair. Nevertheless, its paracrine effects in fibroblasts remain unexplored. Therefore, the present work evaluated the paracrine action of DPCs in skin wound healing in vitro by assessing fibroblast activation and hair regeneration. Fibroblasts were evaluated by culturing with DPCs conditioned medium (DPC-CM) and analyzed for proliferation, migration and fibroblast-myofibroblast conversion. DPC-CM increased fibroblast proliferation by BrdU incorporation without evident morphological changes, and improved fibroblast migration in in vitro wound healing model. Both, proliferation and migration are fundamental processes for the reestablishment of skin homeostasis and subsequent repair. Since myofibroblasts are the primary cells involved in wound contraction and scar formation, fibroblast-myofibroblast conversion was functionally and phenotypically investigated through contraction of fibroblast-populated collagen lattice and α-SMA (a myofibroblast marker) expression, respectively. Increased contraction of fibroblast-populated collagen lattice was observed in the presence of DPC-CM, although no difference in the number of α-SMA-positive cells were detected by immunofluorescence. This result could be explained by the enhanced fibroblast proliferation in collagen lattices promoted by DPC-CM and could be important to regulate wound contraction and scar formation. Furthermore, DPC-CM significantly increased hair growth in organotypic culture of hair follicles. Taken together, these results indicate that DPC-CM could represent a medium rich in growth factors and molecules that regulate hair follicle cycle in situ and also stimulate fibroblast proliferation and migration in addition to fibroblast-myofibroblast conversion and wound contraction. In this sense, DPC-CM may be a promising approach for the treatment of skin wounds, leading to fewer scar formation and hair regeneration at the wound site.

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W-1095

NPY INDUCES HEMATOPOIETIC STEM/PROGENITOR CELL MOBILIZATION BY REGULATING MMP-9 ACTIVITY THROUGH Y1 RECEPTOR IN OSTEOBLASTS

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Hematopoietic stem/progenitor cell (HSPC) mobilization is an essential homeostatic process regulated by the interaction of cellular and molecular components in bone marrow niches. It has been shown by others that neurotransmitters released from the sympathetic nervous system regulate HSPC egress from bone marrow to peripheral blood. In this study we investigate the functional role of neuropeptide Y (NPY) on this process. NPY deficient mice had significantly impaired HSPC mobilization due to increased expression of HSPC maintenance factors by reduction of matrix metalloproteinase-9 (MMP-9) activity in bone marrow. Pharmacological or endogenous elevation of NPY led to decrease of HSPC maintenance factors expression by activating MMP-9 in osteoblasts, resulting in HSPC mobilization. Mice in which the Y1 receptor was deleted in osteoblasts did not exhibit HSPC mobilization by NPY. Furthermore, NPY treatment in ovariectomized mice caused reduction of bone loss due to HSPC mobilization. These results suggest a new role of NPY on HSPC mobilization, as well as the potential therapeutic application of this neuropeptide for stem cell-based therapy.

W-1097

THE NEUROPROTECTION OF HYPOXIC RATS ADIPOSE-DERIVED MESENCHYMAL STEM CELLS(ADMSCS) IN EXPERIMENTAL TRAUMATIC BRAIN INJURY(TBI)

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We previously demonstrated that topical application of adipose tissue-derived mesenchymal stem cells (MSCs) can improve the functional recovery in experimental traumatic brain injury (TBI). In this study, we evaluated whether hypoxic precondition can further enhance the beneficial effects of MSCs. TBI was induced with an electromagnetically controlled cortical impact (CCI) device. 2 million MSCs, derived from the adipose tissue of transgenic green fluorescent protein (GFP)-SD rats and cultured under either normoxic (18% O2) (N=30) or hypoxic (2.5% O2 for 18 hours) (N=30) conditions, were applied to the exposed cerebral cortex within 1 hour after TBI. Neurological functions were evaluated with Water Maze, Roto-rod and Gait Analysis. Animals were sacrificed at days 3, 7 and 14 for microscopic examinations and RT-PCR analysis. Within 3 days following topical application, GFP-positive cells were found in injured brain parenchyma treated with either normoxic or hypoxic MSCs. The rats treated with hypoxic MSCs showed greater significant improvement in neurological functional recovery. In penumbral region, there were significantly ($p < 0.05$) less neuronal death and apoptosis at days 7 and 14 as determined by Cresyl violet and Tunnel Staining respectively. The astrocytosis was suppressed. There was no significant difference in the number of microglia. The expression of pro-inflammatory genes (IL6, IL1a, IL1b, TNFa) and apoptotic gene (caspase 3) were lower. The expression of anti-inflammatory gene (IL10) and anti-apoptotic gene (BAD) were higher. Our study showed that hypoxic precondition of MSCs further enhanced the beneficial effects of MSCs on neurological recovery after TBI.

EYE AND RETINA

W-1099

DERIVATION OF HUMAN RETINAL SHEETS COMPOSED BY CONE PHOTORECEPTORS AND RPE CELLS

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Cone photoreceptors and Retinal Pigmented Epithelium (RPE) are retinal cells required for colors discrimination and high-resolution central vision. Lost in macular degenerations, cone and cone/rod dystrophies, the transplantation of an in vitro engineered retinal sheet could be a therapeutic solution. However, there is no existing protocol. Here, we used human pluripotent stem cells (PSC) to generate (1) human retinal sheets composed by cone photoreceptors in 45 days and (2) human RPE cells that we assemble to form a human retina. Photoreceptors were generated by the constant inhibition of TGFb, BMP and WNT signaling pathways using the human recombinant protein COCO. After 45-60 div, an organized and polarized cellular sheet is formed and can be manipulated. Cellular sheets were transferred on coverslips and stained for photoreceptor markers. More than 90% of the cells were positive for CRX. Confocal microscopy analysis coupled to z-stacks 3D-reconstruction showed 6-7 layers of nuclei with a polarized outer segment positive for PNA and S-Opsin. Further characterization by immunofluorescence and immunoblot revealed the presence of a connecting cilium. Electron microscopy confirmed the presence of an outer segment. RPE cell differentiation were conducted by default PSC differentiation. After 30 div, cells were pigmented and positive for RPE65, ZO1 and BEST1. Taken together, our results show the effective generation of fully differentiated human retinal sheets relevant for cell transplantation. If the coming functional assays are positive, this protocol would enhance the feasibility of retinal cell transplantation.

Funding Source: FFB Canada, Fondation Pierre-Brisebois, University of Montreal.

W-1101

FUNCTIONAL ANALYSIS OF STEM CELL-DERIVED RETINAL PIGMENT EPITHELIAL CELLS FROM AGING RETINAL DEGENERATION PATIENTS

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We have previously generated induced pluripotent stem cells (iPSC) derived from fibroblasts of patients with age-related macular degeneration (AMD) and age-matched controls, and further differentiated them into retinal pigment epithelium (RPE). We report here the functional analysis of these iPSC-derived RPE cells. In short, fibroblasts, grown from skin biopsies, were reprogrammed into iPSCs by Sendai virus technology, while RPE cells were differentiated using our previously published protocol (PMID: 26606685). Newly generated iPSCs and iPSC-derived RPE were characterized at the

molecular level using cell type specific markers. We have assessed trans-epithelial resistance (TER) of RPE cells grown as stable monolayers on transwell plates with a Voltohmmeter and growth factor levels in the supernatants by ELISA. iPSC-RPE cell lines derived from AMD patients and controls formed characteristic monolayers showing typical honeycomb organization and pigmentation. These cells expressed specific RPE cell markers (at the protein and DNA levels) important for differentiation and function. TER analyses showed that dry AMD samples had a significantly reduced TER (29 +/-10 *cm2 (#1); 37 +/-16 *cm2 (#2) and 117 +/-21 *cm2 (#3)) as compared to controls (629 +/-26 *cm2 (control #1); 330 +/- 48 *cm2 (control #2)), while the expression of tight junction genes was conspicuously reduced in AMD samples. As observed in vivo, VEGF and PEDF were secreted preferentially towards the basal and apical RPE sides, respectively. However, the overall secretion of both factors was higher in control samples as compared to AMD, while PEDF showed a higher basal-versus-apical secretion in AMD samples but not in controls. These studies suggest that intrinsic differences may exist in RPE cells derived from AMD patients, which would warrant further investigations. Indeed, studies have shown that the functions explored here are impaired in retinal degenerations including diabetic retinopathy and AMD. Ongoing experiments are aimed at analyzing growth factor and complement activation after challenging iPSC-RPE cells with specific AMD-relevant stressors.

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W-1103

3D TRANSPLANTABLE RPE MICROTISSUE AND THEIR ABILITY TO SUPPORT PHOTORECEPTOR SURVIVAL

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Over 100 million people worldwide are visually impaired due to retinal degenerative diseases such as age-related macular degeneration. This group of diseases leads to irreversible loss of light sensitive photoreceptors and the retinal pigment epithelium (RPE). Current treatments primarily aim to slow photoreceptor loss. In contrast, cell replacement therapies promise to regenerate diseased retinas; however, a major challenge is minimal survival and engraftment of transplanted photoreceptors. This is likely due to the absence of healthy RPE, the layer responsible for providing trophic support to photoreceptors under physiological conditions. To address this obstacle, we are employing micro-scale tissue engineering techniques to develop an effective retinal cell delivery system that improves transplant survival and integration. We hypothesize that the transplantation of engineered retinal micro-tissues will enhance photoreceptor survival, integration and ultimately function. We have successfully generated RPE micro-tissues and are currently characterizing (1) their function by investigating their gene expression profile using RT-PCR and immunohistochemistry for RPE functional genes; (2) the interactions between transplanted micro-tissue and host photoreceptors using live-cell imaging; and (3) photoreceptor survival, maturation, and axon outgrowth using immunocytochemistry, RT-PCR and confocal microscopy. We have discovered that levels of three key trophic factors are enhanced more than thirty-fold in our engineered RPE micro-tissue over conventional adherent cultures. This novel approach will provide an effective retinal cell delivery system that improves the integration of retinal transplants. Overcoming this obstacle is an essential step on the road to correcting the presently irreversible vision loss associated with retinal degenerative diseases.

Funding Source: Brain Canada; ACHRI studentship; Queen Elizabeth II studentship

W-1105

CD200 IS A POTENTIAL NEGATIVE MARKER FOR ISOLATING HUMAN PLURIPOTENT STEM CELL-DERIVED CORNEAL EPITHELIAL CELLS

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We previously reported a new strategy named SEAM-method, for inducing corneal epithelial stem/progenitor cells from human induced pluripotent stem cells (iPSCs) (Hayashi R. et al. Nature 2016, ISSCR 2016). To use iPSC-derived corneal epithelial cells for clinical application, the process for purification of the corneal epithelial cells is required. Particularly removal of undifferentiated cells would be most important because contamination of undifferentiated pluripotent stem cells has a risk for causing side effects such as tumorigenesis after the transplantation to human body. Here we report that CD200 is potential negative marker for purification of corneal epithelial cells. The result of immunostaining for CD200 revealed that CD200 was expressed entirely in undifferentiated iPSC colonies and specifically in neural and retinal zones of differentiated-iPSC colonies. In contrast, no expression of CD200 was detected in corneal and epithelial zones of the differentiated iPSC

as well as in corneal limbal epithelial tissue. After cell sorting of corneal epithelial cells with anti-CD200 antibody, emergence of non-corneal epithelial colony was significantly decreased compared to that in case of using anti-TRA-1-60 antibody, conventional negative marker for corneal epithelial cells. Gene expression analysis demonstrated that expression levels of non-corneal epithelial cells markers including FGFR1 and RPE65 were significantly decreased by using anti-CD200 antibody. In contrast, there was no significant change in corneal epithelial markers. These data indicate that CD200 is a robust negative marker for preventing human iPSC-derived corneal epithelial cells from contamination of unintended cells.

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W-1107

DEVELOPING A CEP290 PATIENT HUMAN INDUCED PLURIPOTENT STEM CELL BASED HIGH THROUGHPUT SCREENING ASSAY TO IDENTIFY SMALL MOLECULE THERAPEUTICS FOR LEBER CONGENITAL AMAUROSIS

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Leber Congenital Amaurosis (LCA) is an autosomal recessive retinal disease that results in severe vision loss at infancy. LCA is most commonly associated with the c.2991+1665A>G deep intronic mutation in CEP290, a gene that encodes a centrosomal protein essential for ciliogenesis in photoreceptors. The c.2991+1665A>G mutation creates a novel splice site which results in a premature stop codon in CEP90 mRNAs that retain the cryptic exon. We successfully removed the CEP290 mutation at high efficiency (>50%) using a Cas9-CRISPR approach. The high repair efficiency suggests that a curative in vivo genome editing may be feasible. An alternative approach may be to target the splicing machinery to effect an increase full-length CEP290 mRNA splice products and CEP290 protein levels. We first tested Madrasin, a commercially available splicing inhibitor, for its ability to normalize CEP290 splicing in undifferentiated hiPSCs. Titrating Madrasin did indeed result in a normalized CEP290 splicing pattern at concentrations that did not alter the splicing of many other genes, including those known to be sensitive to Madrasin. Nevertheless, this compound was toxic and the activity too low to warrant further development. Furthermore, CEP290 protein levels and association with the cilia are more disease-revelant than CEP290 mRNA levels. We therefore developed a high-throughput confocal imaging based assay to determine CEP290 protein levels and subcellular localization. The difference in immunoflurescence phenotpye of WT (or repaired) and mutant CEP290 hiPSCs was most pronounced when dense monolayer areas were imaged at 40x magnification at certain Z level ranges. To increase the speed of imaging we developed a 2-pass imaging scheme wherein each well is first aquired at 2x magnification. Each well image is then analyzed "on the fly" using a high-content imaga analysis script to identify the optimal field(s) for re-imaging at 40x magnification. This platform enabled us to systematically test a small library of highly active molecules targeting different splicing-relevant targets and to perform chemical genetics screens.

NEURAL DEVELOPMENT AND REGENERATION

W-1109

SYNERGIC FUNCTIONS OF MIRNAS DETERMINES NEURONAL FATE OF ADULT NEURAL STEM CELLS

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Adult neurogenesis requires the precise control of neuronal vs. astrocyte lineage determination in neural stem cells. While microRNAs (miRNAs) are critically involved in this step during development, their actions in adult hippocampal stem cells (aNSCs) has been unclear. As entry point to address that question we chose DICER, an endoribonuclease essential for miRNA biogenesis and other RNAi-related processes. By specific ablation of Dicer in aNSCs in vivo and in vitro, we demonstrate that miRNAs are required for the generation of new neurons, but not astrocytes in the adult murine hippocampus. Moreover, we identify eleven miRNAs, of which 9 have not been previously characterized in neurogenesis, that determine neurogenic lineage fate choice of aNSCs, at the expense of astrogliogenesis, Finally, we propose that the 11miRNAs, sustain adult hippocampal neurogenesis

through synergistic modulation of 26 putative targets from different pathways.

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W-1111

THE TRANSITION FROM PROLIFERATING NEURAL STEM CELL TO POST MITOTIC NEURON REGULATES CORTICAL NEUROGENESIS AND NEURONAL FUNCTION

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Deficits in the signaling network regulating the decisions that transform a proliferating stem cell into functional neurons are emerging as contributing to Autism, Rett syndrome, schizophrenia and other neuro-developmental disorders. The precision of the transition from proliferating precursor to a post-mitotic cell destined to become a specific neuron type is well documented in both invertebrate and vertebrate nervous systems. To understand how signaling events in neural stem cells (NSCs), at early time of neurogenesis, regulate the formation of functional neurons, we used FGF2 signaling modulation to control the initial transition of mouse and human dorsal telencephalic precursors into post-mitotic neurons. By using gene expression analysis and bio-informatic tools, we dissected the temporal signaling dynamics of the differentiation and studied the mechanisms linking the proliferation phase with the specification and functionality of neurons, derived from mouse cortical stem cells. We identified distinct classes of neural precursors in this population and show that the neurons were predominantly derived from cells expressing high levels of EGF receptor that respond differentially to endogenous BMP signaling, regulated by FGF2. In addition, temporal activation of BMP signaling in NSCs regulated the efficiency of synaptogenesis in differentiated neurons. Finally, by focusing on sequential dynamics during differentiation, we show NSCs generated by serially passaging human pluripotent stem cells go through distinct cell state transitions, controlled by FGF2 signaling as in the mouse system. In this analysis we specifically defined the gene signatures for the cortical hem, an organizer that patterns the cerebral cortex, whose specification occurs only at early passages, preceding the specification of the cortical neuron precursors. Our data provides novel mechanisms of temporally regulated signaling at early phases of cortical development regulating later neuron differentiation. It will be interesting to use these mechanisms to generate functional models of genetic risk for neurological and psychiatric disorders.

W-1113

ROLE OF NRXN1 IN NEURODEVELOPMENTAL DISEASE: FROM STEM CELL TO CLINICAL PHENOTYPES

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The pre-synaptic protein Neurexin1 (NRXN1) has been implicated in a number of neurodevelopmental diseases including autism spectrum disorder (ASD), However, patient-derived disease models are lacking. Induced pluripotent stem cells (iPSCs) have the potential to revolutionize human disease modelling in vitro, targeting unmet clinical needs. We hypothesize that NRXN1 gene deletion may dysregulate the balance of synaptic excitation and inhibition. NRXN1 is involved in forming synaptic complexes with neuroligins, GABAergic/Glutamatergic receptors and the scaffold proteins SHANKs. Using skin biopsies from patients with NRXN1 deletion and healthy donors, we converted fibroblasts into iPSCs by reprogramming. Their pluripotency was validated by assessing the expression of stem cell markers (OCT4, SOX2 and NANOG). The iPSCs were directed to cortical glutamatergic neurons using a dual SMAD inhibition method. Neural stem cells derived from the iPSCs expressed the neuronal markers of NESTIN, FOXG1 and PAX6. Mature 100-day old neurons were shown to express markers of neuronal maturity (MAP2) and synapses (SYN1) at both the RNA and protein levels. They also transcribed pre- and post-synaptic interaction partners including CASK, MINT, MUNC18-1 and PSD95, which showed no significant difference between patient and control neurons. Unexpectedly, our preliminary data from qRT-PCR revealed an up-regulation of SHANK1 in 100-day old neurons with NRXN1 deletion. We have carried out live cell calcium imaging on 100-day neurons with Fluo4, and neuronal networks displayed inherent spontaneous firing activity.

Additionally, increased calcium spiking was observed in response to extracellular KCl (60mM) and glutamate (30mM) challenge. Our ongoing work will determine how elevated SHANK1 expression may alter the excitatory and inhibitory balance in patient neurons by functional assays. Transcriptome changes are currently under investigation to uncover molecular pathways associated with ASD neurons. To our knowledge this is the first report of iPSC derived mature and functional neurons from NRXN1 deletion patients using dual SMAD inhibition. Further functional characterization will enhance our understanding of the disease cell phenotype towards the development of screening assays for therapeutic intervention.

Funding Source: This study is supported by Science Foundation Ireland Investigator (SFI) award 13/IA/1787.

W-1115

DERIVATION OF SPINAL SENSORY INTERNEURONS THROUGH BMP4 MEDIATED PROGRAMMING OF MOUSE EMBRYONIC STEM CELLS

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Spinal cord injury patients need to be able to recover somatosensation: the ability to experience touch, pain, and heat. These sensory modalities are encoded by an intricate network of dorsal spinal interneurons (dIs) whose diverse identities depend on Bone Morphogenetic Protein (BMP) signaling. However, it remains unresolved how the activities of the BMPs are translated into a variety of dI fates. Directed differentiation of embryonic stem cells (ESCs) offers a unique opportunity to elucidate these mechanisms of dorsal spinal cord development. During embryogenesis, the spinal cord and somatic mesoderm arise from bi-potential neuromesodermal progenitors (NMP) that are positive for Sox2, a neural marker, and Brachyury, a mesodermal marker. Recent studies have shown that mouse (m) ESCs can be directed towards an NMP fate and then differentiated into spinal motor neurons. We have now modified this protocol to direct NMPs towards dorsal interneuron fates. The sequential addition of retinoic acid and BMP4 results in mESCs expressing first spinal- and dorsal-specific markers, such as Hoxa5 and Pax3, and then dI subtype specific progenitor markers, such as Atoh1 and Ascl1. These progenitors eventually differentiate into proprioceptive dI1 and mechano-sensory dI3 neurons. This step-wise progression of ESCs from dorsal spinal progenitors to mature neurons suggests first, that our protocol faithfully recapitulates the in vivo developmental program for generating dorsal interneurons. Second, it permits us to capture different differentiation states of two key classes of dorsal spinal neurons, and thereby investigate the ability of the BMPs to direct these dI fates at a transcriptomic level. An understanding of the intrinsic mechanisms that drive dI fates will ultimately permit us to effectively generate the complete diversity of spinal sensory neurons, needed to successfully regain sensation.

W-1117

TRANSPLANTED HUMAN IPS CELL-DERIVED NEURONAL PRECURSOR CELLS PROMOTE MOTOR FUNCTIONAL RECOVERY AFTER CHRONIC SPINAL CORD INJURY

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Previously we have reported that neural stem/progenitor cells derived from human iPS cells (hiPSC-NS/PCs) pretreated with gamma-secretase inhibitor (GSI), which called human iPS cell-derived neuronal precursor cells (hiPSC-NeuPCs), promoted more neuronal differentiation and maturation in vitro. And in vivo, transplantation of these cells differentiated into more mature neurons without tumorigenicity and maintained greater functional recovery at sub-acute spinal cord injury (SCI) (Okubo et al, Stem Cell Reports 2016). The purpose of the present study was to elucidate the effectiveness of transplanted these cells for chronic SCI in mice. To obtain hiPSC-NeuPCs, safe hiPSC (201B7)-NS/PCs were pretreated with GSI for 1 day before transplantation. We induced contusive SCI at T10 level, and transplanted hiPSC-NeuPCs (NeuPC group), hiPSC-NS/PCs (Control group) or PBS (PBS group) at 42 days after injury. At 89 days after transplantation, immunohistochemical findings revealed that the transplanted cells survived and did not cause tumor-like overgrowth. The proportion of pan-ELAVL positive mature neuron was significantly increased in the NeuPC group, and more growth-associated protein 43-positive fibers were observed, indicating that the axonal regrowth was promoted. Quantitative analysis revealed that the transverse area of the spinal cord at lesion epi-center was significantly decreased in the other groups compared with the NeuPC group, suggesting that the NeuPC group transplantation prevented atrophy of the injured spinal cord. Luxol fast blue staining also revealed a greater preservation of myelinated areas in the NeuPC group compared with the other groups.

The functional recovery was enhanced at 56 days after transplantation and maintained thereafter in the NeuPC group compared with the other groups. These results indicate that only transplantation of hiPSC-NeuPCs differentiated into more mature neurons and maintained functional recovery even at chronic SCI. However, the degree of functional recovery was smaller at chronic phase compared with sub-acute phase. Therefore, we will evaluate efficacy of hiPSC-NeuPC transplantation combined with rehabilitation therapy to enhance greater functional recovery even at chronic SCI.

W-1119

SITE-SPECIFIC MIGRATION AND NEUROLOGICAL DYSFUNCTIONS IMPROVEMENT IN RAT OF NEURAL TUBE DEFECTS BY EARLY INTRA-AMNIOTIC ADMINISTRATION OF MESENCHYMAL STEM CELLS

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Neural tube defects (NTDs) rank among the most common categories of congenital anomalies and available treatments have limited efficacy. Our previous studies have demonstrated that transplanted mesenchymal stem cells (MSCs) into the fetal spinal column in utero could treat neuron deficiency in NTDs rats, but the prenatal surgery for cell transplantation are performed at no earlier than the second trimester, which is already late into the pathophysiological process in NTDs. Here, using the NTDs rat fetal model ex vivo and in vivo, we devised a safe and effective transplantation approach that delivered MSCs into amniotic cavity of early embryos to treat NTDs. Transplanted MSCs showed the capability to specifically migrate into damaged neural tubes, the highest engraftment rate (21.95%) of MSCs was obtained in the embryos with cranial NTDs, while only 0.034% of transplanted MSCs was survived in normal embryos. And using the RNA-seq technique, we identified HGF/MET signaling in focal adhesion pathway was associated with the regulation of MSCs homing to defective neural tubes. In vivo, we further demonstrated engrafted MSCs could differentiate into neurons, glias, epithelia and myocytes depending on the niches they survived, and could enhance the expression of neurotrophic and anti-apoptotic factors in the damaged neural tube. Meanwhile, in the amniotic fluid with MSCs injection, we observed an increased expression of various growth factors and a reduced level of inflammatory factors via protein chip analysis. Finally, electrophysiological and skin lesion analysis demonstrated that as compared with the fetuses with no MSCs injection, a shorter latency (No injection: 25.32 Vs. MSCs injection: 22.82 ms) and a higher MEPs amplitude (No injection: 0.18 Vs. MSCs injection: 0.26 mV) were discovered in the fetuses with MSCs engraftment, and the area of skin lesion area in fetuses with MSCs engraftment was reduced 29.94%. We concluded that intra-amniotic delivery of MSCs was effective and resulted in a clinically relevant motor improvement in neurological function and accelerated skin repair in NTDs rat fetuses via the neural/epithelial regeneration and neuroprotection. Intra-amniotic delivery of MSCs is an innovative platform for developing fetal therapeutics to safely and efficaciously treat congenital diseases.

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W-1121

USING HIPSC-DERIVED NEURONAL CULTURES TO ASSESS THE SAFETY OF PRE-SCREENED POTENTIAL THERAPEUTIC COMPOUNDS AGAINST ZIKA VIRUS

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The recent global threat of Zika Virus epidemic has highlighted the need for sophisticated screening systems capable of detecting unintended toxicity of candidate compounds against this serious infection. Toxicity to the Central Nervous System (CNS) is a key aspect in safety pharmacology evaluation of drugs under development. The characterization of the toxicological profiles of new chemicals to the CNS involves extensive investigation using in vitro and in vivo models. Currently, primary cultures and animal models are popular platforms for those studies. In spite of their importance, those platforms typically are not amenable to larger scale toxicity screens. Human induced pluripotent stem cell (hiPSC) technology has enabled the ready availability of large and consistent batches of neural cells and tissues for wider toxicity screens, having the potential to change the current paradigm in pharmacological research. Through hiPSCs and state-of-the-art differentiation protocols, researchers now have available unlimited source of neural cells, able to mimic early and late stage of human CNS development.

These sophisticated cellular models hold great potential in reducing the time to assess toxicity of developing drugs. Here we investigate the toxicological profile of 29 compounds recently described in the literature as potential therapeutic compounds against Zika Virus infection. hiPSCs-derived neural cells at different developmental stages were challenged with this library of compounds in two-dimensional cultures as well as three-dimensional mini-brain organoids. We observed greater susceptibility of the neural tissues to compound toxicity at early stages of development, and decreasing toxicity as the neuronal cultures mature in vitro. Compounds with the safest profiles were further evaluated in high throughput calcium flux and multi-electrode array assays for assessment of potential functional side effects on the normal function of the CNS. In summary, our work highlights the power of a human CNS model in predicting toxicological profiles of proposed drugs against Zika Virus. Moreover, this system can be applied to investigate the safety profiles on new chemical entities, improving predictivity of clinical outcome and reducing overall drug development costs.

W-1123

KETAMINE INDUCES NEUROAPOPTOSIS POSSIBLY THROUGH ELEVATED INTRACELLULAR CALCIUM/MITOCHONDRIAL FISSION/MICRORNA PATHWAY

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Ketamine, one of the commonly used agents in pediatric anesthesia, has been linked to neurodegeneration and cognitive dysfunction in developing animal models, raising concerns about the safety of anesthesia on young children. Previous studies on developing human neurons derived from embryonic stem cells (ESCs) indicate that ketamine induces neuroapoptosis with the mechanisms largely unknown. This study aims to investigate the effect of ketamine on intracellular calcium level, mitochondrial fission, and microRNA profiles. ESCs were differentiated into neurons following a four-step differentiation protocol. Neuron differentiation efficiency was validated by analysis of neuron-specific marker (beta tubulin III) and developing neuronal marker (doublecortin) expression. The 2-week old neurons were exposed to 100 μM ketamine for 6 to 24 h. Neuroapoptosis was assessed by activated caspase 3 activity assay and TUNEL staining. Intracellular calcium level was analyzed using Fura-2 and Fluo-4 AM staining. Mitochondrial fission was assessed using electron microscopy or confocal microscopy imaging of neuronal mitochondria expressing GFP. miScript miRNA arrays (Qiagen) were used in the initial microRNA target identification studies, which was further confirmed using qRT-PCR. The results showed that 24 h-ketamine exposure induced neuroapoptosis. Intracellular calcium level significantly decreased after acute ketamine exposure and increased after 24 h-exposure to ketamine. Electron microscope images demonstrate the increases of autolysosome and mitochondrial fission in the ketamine-treated neurons. In addition, the mitochondria in the ketamine-treated neurons had significantly lower values of both form factor and aspect ratio, further suggesting an increase in mitochondrial fission. Among 88 microRNAs investigated, let-7a/e, miR-21, miR-23b, miR-28-5p and miR-423-5p were found down-regulated, while miR-96 up-regulated in the neurons treated with ketamine for 6 h. Collectively, our findings indicate that 1) ketamine induces neuroapoptosis possibly through the deregulated intracellular calcium/mitochondrial fission/microRNA pathway, and 2) the use of ESC-derived developing human neurons provides a promising in vitro model for studying anesthetic-induced neurotoxicity in humans.

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W-1125

3D PRINTED NERVE GUIDANCE CONDUITS FOR PERIPHERAL NERVE REGENERATION

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Autologous nerve grafts are the most common choice and gold standard for repairing nerve gaps in the incidence of peripheral nerve injury. However, limited availability of autologous grafts forces scientists to look for alternatives. Synthetic and natural biodegradable polymers have been demonstrated to exhibit promising potentials to repair small nerve gaps (less than 3 cm). In our previous study, we have developed a novel biodegradable elastomer poly(glycerol dodecandioate) (PGD) and its derivatives such as polymer poly(glycerol dodecandioate co-fumarate) (PGDF), and showed that electrospun PGD and PGDF fibers could support proliferation and differentiation of the seeded neural cells derived from mouse embryonic stem cells. 3D printing as a fabrication method exhibits many advantages including short fabrication time and quick turnover from design to fabrication. Even more, it does not require trhe creation of a mold like the traditional fabrication approaches, especially in the case of multiple-channel nerve conduits. Thus, in this study we plan to utilize 3D printing technique to fabricate three-dimensional (3D) nerve guidance conduits from PGD and PGDF. Since PGD and PGDF exhibit elastic mechanical properties similar to soft tissues, we hypothesized that 3D printed nerve guidance conduits will be more suitable for

growing axons from pluripotent stem cell derived motor neurons. However, most polymers synthesized in the lab exist in non-filament form that is not suitable for using with 3D printer directly. Thus we design a novel microextruder to replace original printer head for open source 3D Printer (Reprap Prusa I3). The new setup enables us to print novel 3D nerve conduits from PGD and PGDF. And we can take advantage of this novel 3D printing approach to find the optimal design of nerve conduits for peripheral nerve regeneration. In summary, 3D printed PGD and PGDF nerve guidance conduits will be a versatile platform for testing axon growth of neurons during peripheral nerve regeneration.

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W-1127

DECONSTRUCTING THE CELLULAR BASIS OF THALAMIC NUCLEI SPECIFICATION BY IN VIVO CLONAL ANALYSIS

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The mammalian brain consists of both layered structures (e.g. neocortex) and nuclear structures (e.g. thalamus and hypothalamus). Although significant progress has been made in understanding behavior of neural progenitor cells in the neocortex, little is known about principles governing cell fate specification for structures parceled into nuclei such as the thalamus. Here we applied a MADM (Mosaic Analysis with Double Markers)-based genetic approach for lineage-tracing of single progenitor cells in the thalamus from the onset of thalamic neurogenesis. We show with three driver lines, Gli1-CreERT2, Olig3-CreERT2 and Axin2-CreERT2, that individual radial glial cells (RGCs) in the thalamus generate compositionally diverse daughter cells that can contribute to multiple subsets of discrete nuclei. Clonal analysis of basal progenitor cells (BPs) further suggests that thalamic clones may exhibit different degrees of fate commitment up to the last two cell divisions. Our study reveals distinct modes of thalamic nuclei specification, and provides a framework for studying cellular heterogeneity and connectivity in the thalamus.

NEURAL DISEASE AND DEGENERATION

W-1131

EFFICIENT DERIVATION OF EXPANDABLE NEURAL STEM CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human induced pluripotent stem cells (hiPSCs) provide a unique platform for drug screening, disease modeling and cell therapy. hiPSCs -derived neural stem cells (NSCs) are capable of generating indefinite expansion and subsequent differentiation into the various cell types, which could serve as an unlimited source of cells for neural-related cell-based therapies and disease modeling. Although numerous protocols have been reported for the derivation of NSCs from hiPSCs, these methods based on embryoid body culture are the time-consuming and labor-intensive. Here, we developed a new protocol that could rapidly and efficiently generate expandable neural stem cells from hiPSCs. Integration-free iPS cells were generated from human peripheral blood mononuclear cells (PBMC) using the CytoTune®-iPS Sendai Reprogramming Kit following the manufacture's protocol. The properties of the iPSC were confirmed by standard procedure. NSCs differentiation from iPS cells were carried out using a modified protocol by our company. The iPS cells reprogrammed from PBMCs were positive for pluripotency markers NANOG, OCT4, SOX2 and SSEA4 and exhibited the ability of differentiation into three germ layers. Using these iPS cells, we established an optimal protocol for cardiomyocyte differentiation. Flow cytometry analysis showed more than 95% of the cells were positive for NESTIN, SOX1 and SOX2 staining, 50% of the cells were positive for PAX6 staining, after 7 days of differentiation. Furthermore, the differentiated cells showed highly expandable ability, which is critical for large-scale applications. In this study, one seeded iPS cell yielded about 2×104 viable NSCs after 4 times of passaging. Additionally, these NSCs were capable of being differentiated into neurons, evidenced by the expression of dopaminergic marker TH and the motor neuron markers CHAT. Conclusively, we provided a robust platform for scale-up production of iPS cell-derived NSCs with high purity and expandable ability. These NSCs may be useful for drug screening and disease modeling.

W-1133

LARGE-SCALE AND RAPID PRODUCTION OF FUNCTIONAL ASTROCYTE SUBTYPES FROM HUMAN PLURIPOTENT STEM CELLS

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Recent research on mouse astrocytes has highlighted their central role in the normal development and function of the central nervous system, as well as their potential participation in many pathological conditions. Due to the intrinsic differences between rodents and humans, and the limited availability of primary human fetal or mature post-mortem samples, the direct differentiation of human astrocytes form pluripotent stem cells provides an excellent alternative to uncover the complex function of human astrocytes in pathological conditions. Until now, available protocols for the differentiation of pluripotent cells into astrocytes require extremely long culture (up to 3 months). We have now established a rapid and robust 3D spheroid-based culture protocol for the production of astrocytes in large numbers. By recapitulating normal development, we are able to pattern embryonic stem (ES) and induced pluripotent stem (iPS) cells into brain or spinal cord astrocytes. Rapidly after patterning, stem cells acquire a neuroepithelial fate and express markers appropriate to of either dorsal or ventral identity. Subsequently, following dissociation of the spheres, cells acquire an astrocyte-like morphology and express markers such as CD44, S100b and the more mature marker ALDH1L1. These populations of astrocytes can be expanded and/or cryopreserved, surviving freeze-thaw cycles. As with primary astrocytes, the stem cell- derived astrocytes exhibit the ability to transport glutamate and are able to propagate calcium waves. After a short induction with pro-inflammatory stimuli such as TNFα both spinal cord and brain astrocytes become reactive, a hallmark of traumatic and pathological conditions. The production of large numbers of human astrocytes from patient specific cells and the capability of modulating their phenotype in a biologically relevant manner provide an opportunity to unravel the potential contribution of astrocytes in neurodegenerative and neuropsychiatric diseases. Furthermore, the generation of these astrocytes may lead to a better understanding of astrocyte-neuron interaction, as well as cell autonomous disease phenotypes.

W-1135

DOPAMINE INDUCES OSCILLATORY ACTIVITIES IN HUMAN MIDBRAIN NEURONS WITH PARKIN MUTATIONS

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Locomotor symptoms in Parkinson's disease (PD) are accompanied by widespread oscillatory neuronal activities in basal ganglia. Here, we show that activation of dopamine D1-class receptors elicits a large rhythmic bursting of spontaneous excitatory postsynaptic currents (EPSCs) in midbrain neurons differentiated from induced pluripotent stem cells (iPSC) of PD patients with parkin mutations, but not normal subjects. Overexpression of wild-type parkin, but not its PD-causing mutant, abolishes the oscillatory activities in patient neurons. Dopamine induces a delayed enhancement in the amplitude of spontaneous but not miniature EPSCs, thus increasing quantal content. The results suggest that presynaptic regulation of glutamatergic transmission by dopamine D1-class receptors is significantly potentiated by parkin mutations. The aberrant dopaminergic regulation of presynaptic glutamatergic transmission in patient-specific iPSC-derived midbrain neurons provides a mechanistic clue to PD pathophysiology and demonstrates the usefulness of this model system in understanding how mutations of parkin cause movement symptoms in Parkinson's disease.

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W-1137

ENGINEERING THE RAINBOW OF MOTOR NEURON SUBTYPES TO ENHANCE DISEASE MODELING

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Neurodegenerative diseases affect highly specific cell types in the central nervous system. However, the degree to which current reprogramming or differentiation approaches can specify specific neuronal subtypes remains unclear. Amyotrophic lateral sclerosis (ALS) selectively destroys spinal motor neurons (MNs), which eventually leads to systemic paralysis and death. Within the in vivo pool of somatic MNs there exist

subpopulations of cells with differential sensitivity to disease stimuli. Through direct conversion of fibroblasts in vitro using overexpression of MN-specific transcription factors, we have generated a variety of subtypes of induced motor neurons (iMNs). By altering the cocktail of transcription factors in our reprogramming process, we can direct the motor column identity of our MNs from axial innervating medial motor column (MMC) to limb innervating lateral motor column (LMC). Further, we have recently increased the range of iMNs sizes to sizes commensurate with large putative alpha motor neurons, which display increased in vivo vulnerability during ALS pathology compared to smaller gamma MNs. Finally, retrograde progression of the disease (e.g. posterior to anterior) implicates a role for the HoxC genes, which regulate MN identity along axial positions, in MNs differential sensitivity. By addition of HoxC transcription factors to the reprogramming mix, we are able to impose different Hox codes on iMNs. We observe that posterior codes promote neurotrophic-free survival while enhancing iMN vulnerability to mutant SOD1 expression. These results indicate that transcription factor-based reprogramming methods can be used to generate subtypes of neurons within the motor neuron lineage that differ in their molecular and functional properties. Thus, the transcriptional logic used to specify neural progenitor cells into different neuronal types during development is also capable of converting fibroblasts into different neuronal subtypes with remarkable resolution. Generating the rainbow of MN subtypes will allow us to examine how ALS selectively and sequentially targets various MN populations, while others remain resistant.

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W-1139

BENEFICIAL EFFECTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL (HSC) TRANSPLANTATION IN A MOUSE MODEL OF INFANTILE NEURONAL CEROID LIPOFUSCINOSIS (CLN1); RATIONALE FOR PROMISING HSC GENE THERAPY APPROACHES

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Infantile neuronal ceroid lipofuscinosis (INCL), caused by defects in CLN1 gene coding for palmitoyl protein-thioesterase-1 (PPT1), is the most severe form of NCL, leading to vision loss, dementia, epileptic seizures, loss of motor coordination, culminating in premature death. Reconstitution of brain-resident microglia by the progeny of gene-corrected hematopoietic stem-progenitor cells (HSPCs), transplanted in myeloablated recipients, may represent a promising strategy to obtain widespread distribution of wild type PPT1 in the central nervous system (CNS), potentially improving the clinical benefit for INCL. Here we explored: i) systemic administration of busulfan, an alkylating agent capable of ablating brain resident microglia progenitors, as optimized conditioning to enhance the turnover with progenitors derived from transplanted HSPCs; ii) systemic or intracerebroventricular injection of HSPCs isolated from Ppt1 wild type donors, to identify the route of administration contributing to rapid microglia reconstitution ; iii) phenotypic assessment of transplanted INCL mice to evaluate the effects of the HSPC approach. We confirmed sustained and long-lasting donor-cell chimerism in CNS of busulfan conditioned INCL mice independently from the route of HSPCs administration. Transplanted mice displayed a milder disease progression and partial neuronal protection, as compared to untreated controls. Donor-derived microglia cells were widely distributed throughout the CNS, and displayed high positivity for autofluorescent material. This may suggest an attempt of microglia cells carrying the functional Ppt1 to detoxify the storage and support neuronal microenvironment. This constitute first formal demonstration of potential benefits of HSPC-based approaches in INCL. Based on these results, we started a preclinical safety/efficacy HSC-gene therapy study to investigate the effect of

transplantation with HSPCs transduced with a lentiviral vector allowing expression of supraphysiological levels of human PPT1. We expect that microglia cells derived from the progeny of gene-corrected HSPCs will potentially ameliorate the disease phenotype, by acting as local source of functional enzyme, allowing its sustained and widespread distribution in the CNS. The study is currently in progress.

W-1141

LETHAL GIANT LARVAE 1 (LGL1) PROMOTES ASYMMETRIC CELL DIVISION AND DIFFERENTIATION OF OLIGODENDROCYTE PRECURSOR CELLS

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Embryonic neural stem cells and adult oligodendrocyte progenitor cells (OPC) undergo asymmetric cell division (ACD) to self-renew and generate functional cells. ACD is a fundamental process to restrict proliferation and balance it with self-renewal. Loss of ACD at the expense of symmetric, self-renewing divisions is observed when OPC turn into glioma cells. The majority of ACD regulators in Drosophila neuroblast are conserved in the mammalian genome. Lethal giant larvae 1 (Lgl1) has been implicated in the asymmetric localization of cell fate determinants in neural progenitor cells. Functional characterization of mammalian ACD homologues is incomplete, especially in OPC. The objective of this project is to provide a better understanding of how ACD is established and regulated and to determine if disruption of ACD is causal to neoplastic transformation. To reach this goal, we determine whether Lgl1, a gene that was initially identified as a tumor suppressor in Drosophila, regulates ACD in corpus callosum progenitor cells. We propose that Lgl1 regulates ACD and thereby restricts proliferation and promotes differentiation in OPC. Indeed, in murine OPC carrying conditional null alleles of Lgl1, depletion of Lgl1 in vivo increases symmetric divisions of proliferative NG2+ OPC and disrupts ACD, leading to a decrease in CC1+ pre-myelinating oligodendrocytes cells. In a murine model of spinal cord demyelination, Lgl1 ablation in NG2+ OPC in vivo increases their proliferation during re-myelination but the cells fail to differentiate properly. We confirmed in vitro that Lgl1 loss increases proliferation of OPC but disrupts asymmetric divisions and differentiation. Transcriptome analyses of Lgl1 depleted OPC provide cues into the mechanism by which Lgl1 regulates ACD and will be discussed at the presentation. Our data suggest that loss of Lgl1 disrupts ACD, which contributes to phenotypes associated with malignant transformation.

W-1143

MULTIPLE SCLEROSIS: A DISEASE IN A DISH

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Multiple sclerosis is the most common cause of chronic neurological disability in young adults, despite this the aetiology is poorly understood and this is largely due to the limited availability of suitable tissue and limitations of current models. In order to gain insight on this complicated disease, a model was created that successfully recapitulates some characteristics of the disease. This disease in a dish model compares adipose stem cells obtained from both multiple sclerosis and non-multiple sclerosis patients that have undergone neural differentiation. The cellular proteome was analysed and protein abundance changes quantified by liquid chromatography tandem mass spectrometry, while quantification of secreted cytokines was performed using a Bio-Plex Multiplex immunoassay. This proteomic analysis highlighted the functional complexity of patient derived stem cells, as protein abundance differ both spatially and temporally, consequently providing substantial insight into the phenotypic characteristics of the disease itself. The 27 cytokines that were examined are associated with immune signalling and contribute to neurological functions such as synaptic plasticity. As multiple sclerosis is an autoimmune disease, it is vital to understand the role inflammation has on the fate of stem cells. Cytokine expression in multiple sclerosis patients had greater homogeneity than that of non-multiple sclerosis patients, which was expected as the non-multiple sclerosis patient cohort could have various undiagnosed and undetectable health conditions. Interestingly, cytokine secretions significantly differed between patient cohorts and suggested that adipose stem cells isolated from multiple sclerosis patients have a reduced neurogenic capacity. This was further supported through phase contrast microscopy throughout neuronal differentiation, as the multiple sclerosis patient derived adipose stem cells did not appear to undergo the same morphological changes that are characteristic of differentiation. The data generated from this investigation offers a comprehensive analysis of adipose stem cell neurogenic capacity, and establishes a robust disease in a dish model that reveals crucial insight on multiple sclerosis.

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W-1145

METFORMIN LEADS TO SEX- AND AGE-DEPENDENT EFFECTS ON NEURAL PRECURSOR CELLS AND PROMOTES COGNITIVE RECOVERY IN MOUSE MODELS OF CHILDHOOD BRAIN INJURY

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Metformin (Met), an anti-diabetic drug, enhances the neural precursor cell (NPC) pool and improves spatial memory, suggesting its potential to engage endogenous repair processes in the brain following injury. A population of NPCs are found in the subventricular zone and the niche is altered by factors such as age and sex, which can affect activation and growth of NPCs. Here, we investigated whether these variables would interact with Met, leading to differential effects on NPCs. To investigate age, Met was administered in vitro and in vivo to early postnatal, juvenile and adult mice. Met enhanced the NPC pool in early postnatal, but not older mice. The inhibitory effect of the niche was confirmed by examining Met's effect on pure populations of NPCs (in the absence of the endogenous niche), which led to Met-induced activation of NPCs from all ages. Next, we used a similar approach to investigate the involvement of sex. NPCs from early postnatal males and females were both responsive to Met; however, only female NPCs responded to Met in the adult. Again, removal of the endogenous niche resulted in increased responsiveness of male NPCs. Together, these data suggest an inhibitory effect of both the male and adult niches. Considering these variable effects of Met treatment, we investigated its potential therapeutic effect in two mouse models of childhood brain injury that lead to cognitive impairment. Early postnatal mice were subjected to hypoxia-ischemia (H/I), then received Met for 5 weeks. Memory and executive function were assessed at 7 weeks post-injury. H/I resulted in a learning impairment which was completely rescued with Met treatment in females, coincident with our cellular data. Met treatment was then investigated using a second injury model, cranial irradiation (IR). Juvenile mice received IR followed by 25 days of Met. IR alone resulted in sex-dependent cognitive deficits. As in the H/I model, Met treatment improved cognition in females only. Taken together, these findings reveal that 1) the stem cell niche plays a role in the response of NPCs to Met, and 2) Met is able to differentially rescue cognitive function following brain injury. This highlights various considerations and limitations of applying activation strategies for neural repair, a crucial consideration in translation to clinical applications.

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W-1147

TRANSCRIPTOME ANALYSIS IN FUS MUTANT IPSC-DERIVED MOTOR NEURONS REVEALS ALTERED PATHWAYS IN AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease due to loss of motor neurons (MNs). A subset of familial ALS cases is linked to mutations in the FUS gene, however the molecular pathways leading to MNs death are still poorly understood. Induced pluripotent stem cells (iPSCs) provide an opportunity to study human diseases when no proper model systems are available. We have recently derived different human iPSC lines carrying ALS mutations in the FUS gene, either by reprogramming or by gene editing. Notably, mutant iPSC lines generated by gene editing could be compared to isogenic control lines, avoiding any bias due to the genetic background. We have previously shown that this system represents a suitable platform to model FUS-related ALS in vitro. We are taking advantage of this tool to unravel the deregulated pathways in human FUS mutant MNs. Coupling a rapid and high yield differentiation protocol with a fluorescent reporter stably integrated by genome editing, this system allowed isolation of pure populations of human MNs by FACS. We performed transcriptome analysis by next generation sequencing from WT and FUS-P525L MNs. Data analysis revealed several deregulated transcripts and gene ontology analysis highlighted relevant categories that cooperate in pathways and distinct molecular functions, such as those related to "cell-cell adhesion" and "calcium ion binding". We are currently exploring the possible contribution of these altered pathways in MNs survival. Since FUS is an RNA-binding protein linked to multiple aspects of RNA metabolism, we are investigating the molecular mechanisms by which mutant FUS alters gene expression. To identify targets directly regulated by FUS, we are performing PAR-CLIP assays in MNs. Collectively, our ALS FUS iPSC model system provides

interesting insights into the molecular pathways altered in ALS pathology.

Funding Source: AriSLA - Fondazione Italiana di Ricerca per la SLA; Istituto Italiano di Tecnologia; Sapienza University

W-1149

MODELING ASPECTS OF BIPOLAR DISORDER IN NEURONS AND ASTROCYTE FROM PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

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Bipolar Disorder (BD) is a genetically heterogeneous psychiatric disorder of unknown etiology that presents major challenges for the study of disease biology and drug development. Induced pluripotent stem cells (iPSCs), which can be differentiated into neurons and glia, provide a cellular model system suitable for studies of cases and controls as well as studies that assess the impact of therapeutic agents on cellular development and differentiation. As part of the Amish-Mennonite Bipolar Genetics study (AMBiGen), we are collecting clinical data, DNA, and fibroblasts from probands diagnosed with BD and their relatives, all ascertained within genetically-isolated Amish and Mennonite communities in the Americas. Fibroblasts have been reprogrammed to iPSCs with Sendai virus and differentiated into neural progenitor cells (NPCs), neurons, and astrocytes using standard protocols. Pilot studies are underway in cells from 4 probands and 4 sex-matched, unaffected siblings (2 clones each). Assessments include morphology, action potentials, gene expression profiles, and cellular response to therapeutic dosages of established treatments such as lithium and valproic acid (VPA). Preliminary results suggest that long-term treatment with VPA, but not lithium, greatly reduced proliferation of NPCs by the MTT assay, and promoted neuronal differentiation in both cases and controls. Long-term treatment with lithium greatly reduced neuronal calcium response to glutamate stimulation in both cases and controls, based on single-cell calcium imaging. Interestingly, differentiation of NPCs into astrocytes was substantially slower and doubling time was shorter in cells derived from cases. VPA treatment (10 d at 1 mM) significantly reduced astrocyte growth in cells derived from both cases and controls, but the impact of VPA was substantially greater in case-derived cells. Lithium treatment (10 d at 1 mM) increased astrocyte density in lines derived from healthy controls, but not in lines derived from cases. These preliminary results, if replicated, could point to cell-autonomous phenotypes detectable in astrocytes derived from people diagnosed with BD. If astrocyte development is impaired in BD, this could have important implications for research into etiology and therapeutics.

W-1151

THE EFFECTS OF DIFFERENT TYPES OF PRENATAL IMMUNE ACTIVATION EVENTS ON NEOCORTICAL NEURAL STEM AND PROGENITOR CELL PROLIFERATION AND MAINTENANCE

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Maternal illness during early pregnancy has been shown to increase offspring's risk for autism spectrum disorder (ASD). Increasing evidence from human epidemiology and twin studies suggests that both genetic and environmental risk factors contribute to the etiology of ASD. Among many environmental risk factors, systematic immune disturbance induced by maternal immune activation (MIA), events such as prenatal infection is known to be the most relevant ASD risk. However, the similarities and differences of pregnancy outcomes resulting from different prenatal MIA challenges has not been fully elucidated. Here, we studied the distinct effects of two different MIA events on neocortical neural stem and progenitor cells (NPCs) in the developing fetus and particular behavioral domains in postnatal offspring. We specifically focused on bacteria-like and virus-like MIA responses by triggering toll-like receptor (TLR)-mediated signaling pathways. Common environmental pathogens and other innate immune activators act directly on TLR pathways to initiate an innate immune response. The various TLR-signaling pathways have been shown to elicit distinct immune profiles and may evoke discrete pathophysiology associated with ASD at the fetal-maternal interface during the time course of infections. Pregnant mice were challenged with TLR3- and TLR4-specific agonists during mid-gestation, and the pregnancy outcomes and alterations in NPC behaviors following MIA challenges were assessed. Differences in cytokine and chemokine profiles were detected in maternal serum and the placenta following MIA challenges compared to controls. An increase in hypoxia in the fetal brain was also observed. Impairments in neocortical NPC cell cycle,

proliferation, and cortical patterning in the neocortex were found in the MIA-challenged fetuses. The MIA-challenged postnatal offspring displayed behavioral deficits such as alterations in social interactions and an increase in stereotyped behaviors. Taken together, we identified impairments in neocortical NPC proliferation and maintenance during early brain development which ultimately may lead to pathophysiology and postnatal behavioral deficits relevant to ASD.

W-1153

IN VITRO DISEASE MODELING OF AMYOTROPHIC LATERAL SCLEROSIS: IMPAIRMENT OF MICRORNA FUNCTION IN HUMAN MOTONEURONS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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Mutations in the RNA-binding protein FUS are genetically linked to Amyotrophic Lateral Sclerosis (ALS), a neurodegenerative disease primarily caused by the death of motoneurons (MNs). Most of ALS mutations impair FUS nuclear localization and trigger its aggregation in cytoplasmic insoluble inclusions. Previous studies in cell lines and animal models showed that FUS is a ubiquitous protein involved in mRNA metabolism processes (including transcription, splicing, transport and localization) and in microRNA (miRNA) processing. Conceivably, the set of FUS RNA targets is context-dependent and a comprehensive transcriptome analysis in cell types relevant for ALS is still missing. In particular, a link between FUS mutations and miRNA function has been hypothesized, but never verified, in human MNs. Human induced Pluripotent Stem Cells (iPSCs) carrying pathogenic mutations represent a powerful tool in neurodegenerative disease modeling. We took advantage of ALS-iPSC lines recently raised in our lab to address, for the first time in human MNs: a) whether ALS mutations in FUS affect the miRNA pathway; b) which miRNAs are specifically under FUS control; c) what are the consequences of miRNA dysregulation. We obtained pure populations of human MNs, either FUS-WT or isogenic FUS mutants generated by gene editing. RNA collected from these samples was used for Next Generation Sequencing (NGS) to analyze coding and non-coding RNAs, including small RNAs. We identified a subset of MN miRNAs differentially expressed in the FUS mutant, possibly by a pathological loss of nuclear function. Interestingly, these include miRNAs previously associated with MN survival as well as other miRNAs that could potentially target genes relevant for ALS. Interestingly, genes involved in apoptosis are among miRNA targets dysregulated in mutant MNs. Our results suggest a novel possible pathological mechanism underlying FUS ALS pathogenesis.

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W-1155

DYNAMIC REMODELING OF NEURAL MICROENVIRONMENT SIGNATURES DEPICTED IN 3D IN VITRO DIFFERENTIATION OF HUMAN IPSC-DERIVED NSC

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Brain microenvironment plays an important role in neurodevelopment and function. Here, extracellular matrix (ECM) components and soluble factors can modulate important cellular features, as migration, proliferation survival and neuronal function. Disruption of this microenvironment's homeostasis is often related to pathological conditions. We hypothesized that 3D differentiation of human neural stem cells (NSC) as neurospheres in perfusion stirred-tank bioreactors can sustain microenvironment remodeling, enabling to address key cell-ECM interactions in healthy and pathological settings. Differentiation of hiPSC-derived NSC was shown to recapitulate neurogenic developmental pathways, generating tissue-like 3D structures with neuronal, astroglial and oligodendroglial cells. Changes in neural microenvironment during neural differentiation, namely at cell membrane and ECM composition, were addressed using quantitative transcriptomic (NGS) and proteomic (SWATH-MS) data. This revealed a significant divergence between neurosphere or monolayer (2D) differentiation. Structural proteoglycans, such as neurocan, versican, brevican and tenascin C, were significantly enriched relative to 2D. Inversely, basement membrane constituents (e.g., laminins, collagens and fibrillins) were found to be downregulated in the differentiated neurospheres, while enriched in 2D. Moreover, a clear increase in the expression of important synaptic machinery constituents was observed in 3D, both at mRNA and protein levels, suggesting a higher degree of neuronal maturation and organization. In summary, we demonstrated that cellular and extracellular developmental features are recapitulated in the presented neural cell model. This strategy is currently being employed to address

molecular defects associated with neurological disorders affecting the microenvironment homeostasis, such as the lysosomal storage disease mucopolysaccharidosis type VII.

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CANCERS

W-1159

ESTABLISHMENT OF PATIENT-DERIVED CD133+ STEM-LIKE CANCER CELLS FROM AN ATYPICAL MENINGIOMA

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Although meningioma are the most common form of primary central nervous system (CNS) tumors, investigations of meningioma biology have come to a stop due to the lack of suitable preclinical in vitro and in vivo model systems. In this study, we report the establishment and comparative characterization of patient-derived, spontaneously immortalized cancer cell lines derived from 4 typical (WHO grade I) and an atypical (WHO grade II) meningiomas. Without any immortalization, almost of the established cell lines possess a marked capacity for proliferation due to the endogenous expression of the telomerase catalytic subunit (hTERT). All of the meningioma-derived cancer cell lines showed homogenous Vimentin expression, whereas various expression patterns were examined in Nestin depending on the patients. Western blotting revealed the expression of stem cell surface marker, CD133, but not Nestin as properties of more aggressive atypical meningioma cell line. Interestingly, tumor spheres only generated from the specimen of high-grade atypical meningioma cell line indicating that this cells in spheres were capable of self-renewal and proliferation. The loss of a Y chromosome was examined in 4 out of 30 metaphases (13.3%) obtained from an atypical meningioma cell line. The patient derived cancer cell lines established in this study might be used as a model for meningioma tumorigenesis to improve the success in the clinic by explaining the slightly different clinical behavior among the patients.

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W-1161

PARACRINE SIGNALS PRODUCED FROM CANCER CELLS PROMOTE BONE MARROW MESENCHYMAL STEM CELLS TO UNDERGO REPROGRAMMING TO A CANCER STEM CELL PHENOTYPE

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Mesenchymal stem cells (MSCs) play different roles in modulating tumor progression, growth, and metastasis. Recently, it has been suggested that cellular plasticity within a tumor causes a bidirectional conversion between cancer stem cells (CSCs) and non-CSCs. Although MSCs have been found to be recruited to the tumor site, the effect of the tumor microenvironment on MSC plasticity, remains poorly understood. Herein, we report a paracrine effect in which cancer cells secrete soluble factors, without direct cell-cell contact, that promote a CSC phenotype in bone marrow MSCs (BM-MSCs). The interaction between cancer cells and BM-MSCs generated sphere-like cells that display many properties of CSCs, and accordingly termed cancer induced stem cells (CiSCs). CiSCs expressed many markers of cancer cells, CSCs and pluripotent stem cells, and were able to maintain their pluripotent state and self-renewal capacities in culture. They displayed properties of a side-population and were resistant to chemotherapeutic agents. Interestingly, these cells were quiescent and slow cycling and showed a high resistant to DNA damage. CiSCs also showed enhanced tumorsphere formation capacities in culture, potent invasive properties, were able to form colonies in suspension from single cells and displayed other CSCs features. In line with the recent reports that the EMT process is tightly linked with the function and generation of CSCs, our results indicate that CiSCs were more mesenchymal than their parental BM-MSCs. Microenvironmental stimulation with TGF-β further stimulated the cancerous properties of CiSCs and increased their tumorsphere formation and invasion properties. CiSCs also showed their multilineage differentiation capacities and formed complex secondary structures when cultured on matrigel in a 3D culture system. Ultrastructural analysis of CiSCs using electron microscopy revealed an irregular surface of CiSCs and tumor-like buds on their surface. These findings demonstrate that MSCs recruited in the tumor microenvironment may display cellular plasticity and convert to CSC-like cells. These cells can be generated in large-scale to be used for screening for candidate anti-cancer therapies. Our observations may also hold

promise to generation of patient-specific CSCs without any genetic manipulations.

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W-1163

TARGETING FRIZZLED-7 IN GASTRIC CANCER

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Gastric cancer is a common malignancy of the gastrointestinal tract and is the 2nd most common form of cancer related death. Gastric cancer can be divided into two broad groups; intestinal-type and diffuse-type, as classified by the Lauren system. Of the documented genetic lesions in intestinal-type gastric cancer, several components of the Wnt pathway are frequently mutated, namely members of the Wnt receptor complex. As such, targeting the Wnt receptor complex via Frizzled (Fzd) receptors may provide potential therapeutic benefit in models of intestinal-type gastric cancer. We sought to determine the function and therapeutic benefit of targeting Fzd receptors in pre-clinical models of gastric cancer. Preliminary experiments treating human gastric cancer cells with an anti-Fzd blocking antibody (Vantictumab) reveal significant cell proliferation arrest and a decrease in Wnt activity and associated gene transcription. These in vitro findings were extended in two different mouse models of intestinal-type gastric cancer (gp130FF and Tff1Cre+; Apcfl/fl), demonstrating significant reduction in tumour burden following treatment with Vantictumab. Tumour organoids derived from Lgr5GFP/+; gp130FF mice infected with an adenoviral Wnt-active reporter reveal an overlap between active Wnt signalling and Lgr5+ cells in gastric tumour organoids, which was reduced following treatment with Vantictumab, demonstrating that Fzd inhibition in Wnt-active Lgr5+ cells leads to reduced tumour organoid growth. Of the five Fzd receptors inhibited by Vanticumab (1, 2, 5, 7 and 8), the expression of Fzd7 was found to be the most downregulated compared to other Fzd receptors. As such, we conditionally deleted Fzd7 from mouse models (described above), which significantly reduced overall in vivo tumour burden characterised by significant reductions in cell proliferation, Wnt target gene expression and angiogenesis. Our results show that Fzd7 is critical for transmitting Wnt signals to Lgr5+ cells in gastric tumours, which are likely to fuel tumour progression by providing oncogenic cues to cells. Furthermore, we demonstrate that Vantictumab has significant therapeutic utility in pre-clinical models of intestinal-type gastric cancer and suggest a translational clinical benefit to gastric cancer patients.

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W-1165

CHEMICAL SCREENING IN ZEBRAFISH EMBRYO CULTURES IDENTIFIES RETINOIC ACID AS A TRANSCRIPTIONAL SUPPRESSOR OF MYB AND LEADS TO A NEW TREATMENT FOR MYB-DRIVEN ADENOID CYSTIC CARCINOMA

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MYB translocations, and thus overexpression, are molecular hallmarks of adenoid cystic carcinoma (ACC), a salivary gland tumor. Surgical resection, whenever possible, is the standard therapy for ACC, but there are no available therapeutic options available if surgery fails. Here, we used a novel culture system to find chemical suppressors of a BAC transgenic reporter with GFP at the ATG of the myb gene. By adapting an embryonic blastomere culture system in zebrafish, we screened 3,840 small molecules for suppression of c-myb:GFP expressed in blood cells and neural tissues. Retinoic acid agonists were potent suppressors of c-myb:GFP expression in the zebrafish embryo cell cultures. We confirmed by in situ hybridization that retinoic acids downregulate c-myb positive cells in wildtype zebrafish whole embryos treated between 48 and 72 hours post fertilization. Retinoic acids significantly downregulated c-myb expression in U937 cells, a human leukemia cell line, within 3 hours, likely suggestive of a direct transcriptional mechanism of regulation as retinoic acid receptors bind physically to the MYB locus by ChIP-seq. Since MYB translocations in ACC retain MYB regulatory regions, we reasoned that transcriptional suppression of MYB by retinoic acid would be potentially useful in treating ACC. Patient derived xenograft studies in mice showed an average tumor size inhibition after 28 days of treatment across three primary ACC models of 88% for all-trans retinoic acid and 86% for isotretinoin, which are among the highest levels for any drugs tested in these xenograft models. Translocations involving MYB have been previously described in ACC for bringing strong enhancers into close proximity of the MYB locus, and these translocated enhancers are also bound by MYB protein, resulting in a positive feedback loop

that drives MYB overexpression. ChIP-seq analysis of the xenografts revealed that all-trans retinoic acid treatment significantly decreased MYB binding at these translocated enhancers. We propose that retinoic acids act via retinoic acid receptors to directly suppress MYB expression, thereby disrupting the oncogenic MYB feedback loop at the translocated enhancers that drive ACC. Our findings identify an important role of retinoic acid in MYB regulation and will lead to a clinical trial for ACC shortly.

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W-1167

USE OF NEURAL STEM CELLS FOR TARGETED ONCOLYTIC VIROTHERAPY IN PRECLINICAL MODELS OF STAGE III OVARIAN CANCER

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Ovarian cancer is the most lethal of all gynecologic malignancies with over 22,000 new cases and 14,200 deaths per year in the U.S. Once metastasized to the abdominal cavity (stage III), patients have only a 34% 5-year survival rate following standard surgical debulking and combination chemotherapy. Use of intraperitoneal (IP) chemo regimens has improved outcomes, but due to increased complications and toxicities render most patients unable to complete the treatment cycles. More effective approaches for treating recurrent and/or drug-resistant ovarian cancer are urgently needed. Oncolytic virotherapy is a promising novel approach that uses replication-competent viruses to induce cancer cell death. Success in the clinic has been hampered by rapid immune-mediated clearance/neutralization of the virus, and poor viral distribution to tumor foci. To overcome these barriers, we are using a tumor-tropic NSC line that has demonstrated clinical safety and non-immunogenicity in first-in-human glioma trials. These NSCs have also demonstrated localization to peritoneal ovarian cancer metastases in mice after IP injection. We engineered our NSCs to produce a conditionally replication-competent adenovirus, CRAd-S-pk7, driven by the survivin promoter, which is highly expressed in ovarian cancer, but not in normal differentiated cells. We hypothesize the NSCs will shield the virus from immune recognition on route to tumor, and afford an unprecedented ability to seed and penetrate multiple tumor sites, allowing for amplification of viral payloads in situ. NSG mice with established ovarian mets were injected IP with PBS, cisplatin, CRAd-S-pk7 NSCs, or a combination of cisplatin and CRAd-S-pk7 NSCs. The combination treatment group resulted in significantly decreased tumor burden, as measured by bioluminescence imaging. In vitro data supports in vivo results. Studies underway include free virus distribution pharmacokinetic comparisons, and long-term survival. This novel NSC-oncolytic virotherapy approach can potentially increase survival, reduce toxicities, and improve quality of life for patients with stage III ovarian cancer.

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W-1169

DIFFERENTIAL GENE EXPRESSION SIGNATURE OF CANCER STEM CELLS IN HUMAN COLORECTAL CANCER

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Cancer stem cells (CSC) are believed to be responsible for invasion and metastasis in colorectal cancer (CRC) but the exact mechanism is still unknown. We aim to study the differences between CSC of different grades of CRC to understand their role in metastasis. This would help to identify critical targets for clinical intervention to halt the progression and metastasis of CRC. Paraffin embedded tissue samples of different histopathological grades of primary, untreated CRC and appropriate controls from 70 patients were analyzed for the expression of CSC markers, CD44 and CD166 using immunohistochemistry. Marker based isolation of CSC and non-CSC cells from fresh CRC tissue and human CRC cell lines was done using FACS. Tumor sphere assay was performed with the sorted subsets. Microarray was done using whole genome 4X44K array slides to study transcriptomic changes between CSC and non-CSC cells for both high grade (HG) & low grade (LG) CRC. Data was analysed using Flow Jo, GeneSpringGX 13 and GeneGOMetaCore. Validation was done using Real time PCR. FACS showed higher prevalence of CSCs in primary high grade CRC as compared to low grade CRC. Sorted and cultured CSCs formed tumor spheres. High throughput gene expression analysis of

CSCs showed over expression of the classical stemness markers including Oct4, nanog, c-myc, klf4, MSH1 as well as EMT markers including MMPs, Snail, Twist and ZEB1. Gene expression profile of CSCs from HG and LG tumors were found to be different. Posthoc analysis (ANOVA with Tukey with Bonferroni FWER, $p < 0.05$, fold change >2) revealed a total of 495, 481, 202, 119 genes differentially expressed between CSC from HG and LG, non-CSC from HG and LG, CSC and non-CSC from HG, CSC and non-CSC from LG respectively. A unique gene signature representing CSC and non-CSC in LG and HG CRC was computed and validated in arbitrarily selected 10 genes. Networks and enrichment analysis revealed different metabolic pathways active in CSC and enrichment of survival, ECM- cell interaction and cell adhesion pathways. We hypothesize that the high metastatic potential of HG CRC may be accredited to the differential expression profile of CSCs. Our study identified novel gene signatures and pathways for high grade CSC which may be manipulated to target CSC in aggressive CRC.

W-1171

THE BMP4-SHH CROSSTALK CONTROLS MALIGNANCY AND CHEMORESISTANCE IN ESOPHAGEAL CANCER BY MODULATING STEM CELL FUNCTION

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BMP4 is a growth factor with a key role in carcinogenesis and metastasis. In Gastro-Intestinal cancers presenting mutations in the canonical transcription factor SMAD4, BMP4 induces tumorigenesis through activation of non-canonical signaling pathways. Our recent studies have demonstrated a role of BMP4 as a positive regulator of chemo-resistance and invasiveness in EAC, and suggest that inhibition of BMP4 with highly specific antibodies ameliorate the malignant behavior of aggressive SMAD4 negative esophageal adenocarcinoma (EAC). BMP4 controls quiescence and differentiation of stem cells. Whether BMP4 controls malignancy in EAC through modulating cancer stem cell function is not known. To determine the role of BMP4 in EAC stem cells, we analyzed the RNA expression profile of tumor biopsies of 74 EAC patients. An aggressive BMP high gene signature profile which correlated to poor survival and recurrence was identified. This gene signature is characterized by the expression of BMP ligands as well as their receptors. Expression of BMP4 was also correlated Sonic Hedgehog (Shh), which is the BMP upstream ligand and a known regulator of stem cell function. Interestingly, both BMP4 and Shh correlated to the expression of several stem cell markers such as lgr5, HNF4A, and SOX9, suggesting a possible role for the BMP4/Shh crosstalk in stem cell function. To further examine the malignant role of this crosstalk, we applied the BMP/Shh signature to a panel of EAC cell lines and demonstrated that in a SMAD4 mutated cell line, it correlated with chemoresistance, migration as well as stem cell proliferation. Further, in vitro analyses revealed that Shh signaling on epithelial cells activated autocrine BMP4 secretion which resulted in an increase in the malignant features of EAC cancer cells, such as chemoresistance as well as the invasive capacities. Most importantly, these malignant features could be inhibited by our recently developed novel anti-BMP4 antibodies. Together these results show how the BMP4/shh crosstalk can modulate malignant features in EAC with SMAD4 mutations by controlling stem cell function. Of importance is the finding that specific anti-BMP4 antibodies can inhibit these effects in vitro and they might represent a novel therapy in targeting stem cell function in EAC.

Funding Source: Targets4Barretts (ERC Starting Grant)

CHROMATIN AND EPIGENETICS

W-1175

THE HISTONE DEACETYLASE SIRT6 REGULATES TRANSCRIPTION ELONGATION BY SUPPORTING RNA POLYMERASE II PROMOTER-PROXIMAL PAUSING IN EMBRYONIC STEM CELLS

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How chromatin dynamics regulate transcription to influence embryonic stem cell (ESC) function remains a major question. The histone deacetylase SIRT6 targets histone H3 at lysine's 9 (H3K9ac) and 56 (H3K56ac) to regulate glucose metabolism and cell fate lineage specifications in ESCs. Earlier, we found SIRT6 to suppress expression of metabolic and pluripotent genes at the mRNA level. However, the mechanisms underlying SIRT6-dependent transcriptional control remain unclear. A key step during transcription regulation occurs via promoter-proximal pausing of RNA polymerase II (Pol II), which prevents transcription elongation. Chromatin accessibility via histone acetylation accelerates transcription elongation, however the histone deacetylase(s) modulating this step are unknown. We found co-localization of SIRT6 and Pol II at promoter-

proximal regions in ESCs genome wide. In addition to a global increase in H3K9ac and H3K56ac, we found phosphorylation of the carboxy-terminal domain of Pol II at serine 2, a hallmark of transcription elongation, to be elevated in ESCs derived from Sirt6 knockout (S6KO) mice, suggesting SIRT6 as a repressor of transcription elongation. We demonstrate SIRT6-dependent Pol II pausing by permanganate DNA footprinting and Pol II ChIP-Seq analysis. Moreover, while the negative transcription elongation factor NELE-e is evicted from chromatin, the positive transcription elongation proteins BRD4, Myc and the Pol II-associated protein 1 (PAF1) accumulate in chromatin fractions of S6KO ESCs. Concordantly, siRNA-mediated knockdown of BRD4, Myc or PAF1 rescues the elevated expression of metabolic genes in S6KO ESCs. Lastly, mehylation of histone H3 at lysine's 36 (H3K36me3) and 79 (H3K79me2), hallmarks of transcription elongation, parallel the expression of metabolic and developmental genes, which are upregulated in S6KO ESCs. Thus, we unraveled a new mechanism involving SIRT6-dependent histone deacetylation as a key regulator of transcription elongation by sustaining Pol II promoter-proximal pausing in ESCs affecting genes implicated in metabolism and early development.

W-1177

DEVELOPMENT OF A NOVEL ENHANCER PREDICTION MODEL USING TRANSCRIPTION FACTOR BINDING SEQUENCES OCCUPIED IN EMBRYONIC STEM CELLS AND CONSERVED AMONG MAMMALS

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Enhancers regulate tissue specific gene expression and play a major role in evolution, disease and development. As a result there is great interest in identifying enhancers; however, it has been a challenge due to their variable location from their target gene, low overall sequence conservation compared to genes and lower precision of bioinformatics models based on histone modification alone. To develop a novel sequence based bioinformatics model, we first identified positive enhancers as Multiple Transcription factor bound Loci (MTL) using the ChIP-seq data of nine transcription factors (TFs) in mouse embryonic stem (ES) cells. MTL regions bound by increasing numbers of TFs (MTL2-4 and MTL≥5) were found to have increased enrichment for enhancer features, histone H3 K27 acetylation (H3K27ac) and EP300, and were associated with higher expression of nearby genes. Liftover of MTL2-4 and MTL≥5 to the human genome identified 432 regions with conserved enhancer features in both species. Enhancer function was confirmed by luciferase reporter assays for a subset of these regions. Clover analysis on these enhancers using the JASPAR database revealed transcription factor binding sites (TFBS) overrepresented in both mouse and human. TFBS conservation in the corresponding human and mouse enhancers was analyzed using multi-sequence alignment (MSA). Average TFBS conservation between mouse and human was found to be highest for OCT4:SOX2 dimers (89%), while additional important ES cell expressed TFs including: KLF4, ESRRB, STAT3 and TCFCP2L1 were found to have lower average TFBS conservation ranging between 67-73%. Interestingly, many novel TFs, which have not been previously linked to pluripotency maintenance, were found to have higher TFBS conservation (74-89%). Correspondence analysis of the 432 enhancers using 122 overrepresented TFBS revealed 86 TFBS to have a co-occurrence pattern in enhancers similar to the OCT4:SOX2 TFBS. A novel enhancer prediction model will be developed for ES cells based on multiple occurrences of conserved TFBS within 1000 bp windows in mouse and human genomes. In addition, this approach could be applied to additional cell types, which have a less well-characterized regulatory network to discover novel enhancers and regulatory TFs.

W-1179

DISSECTION OF SUPER-ENHANCER HIERARCHY BASED ON CHROMATIN INTERACTIONS

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Super-enhancers (SEs) are groups of putative enhancers with unusually strong enrichment for mediator binding. Previous studies have shown that SE-associated genes are enriched for master regulators for maintenance of cell identity. However, it remains unclear to what extent SEs are structurally and functionally distinct from conventional enhancers. We hypothesize that many super-enhancers are composed of a hierarchy of constituent elements whose functional difference is associated with long-range chromatin interaction patterns. To test this hypothesis, we developed a systematic approach to classify SEs into hierarchical and non-hierarchical subtypes by integrating Hi-C and ChIPseq data in ESC and differentiated cell types. We find that, comparing with non-hierarchical SEs, hierarchical SEs enriched with pluripotent regulators in ESCs, but with cell-type specific regulators in the differentiated cells. Furthermore, within each hierarchical SE, we found that the hub enhancers are highly enriched for disease-associated variants, which might suggest

their functional potency. In a few cases, the hierarchy within a SE has been previously mapped out by using CRISPR/Cas9-mediated. Comparison between this functional hierarchy and that predicted from chromatin interactions shows strong agreement. Taken together, these results strongly support our hypothesis that many SEs are composed of a hierarchical structure that is associated with chromatin interaction patterns.

ORGANOIDS

W-1183

IN VITRO ASSESSMENT OF DRUG EFFECTS ON HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIAC SPHEROID CULTURES

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There is increasing interest in exploring the use of three-dimensional (3D) cell culture for modeling developmental and tissue biology with the goal of accelerating translational research. Such 3D models can provide different perspectives from traditional 2D cultures on the responses of cells and tissues to drug treatments. Accordingly, the development of quantitative assays in higher throughput using 3D cultures is an important area of investigation. In this study, we developed methods for the formation of 3D cardiac spheroids using human iPSC-derived cardiomyocytes (iCell Cardiomyocytes2). We used high content and fast kinetic fluorescence imaging (FLIPR) to measure the impact of various compounds on the beating patterns and rates of cardiac spheroids as monitored by changes in intracellular Ca2+ levels with calcium-sensitive dyes. We tested a set of known cardioactive and cardiotoxic compounds, including alpha- and beta- blockers, cardiac glycosides, ion channel blockers, anti-cancer drugs, and compounds with other mechanisms of action. This assay was optimized for HTS in 384-well plates and allows for the characterization of cardiac spheroid beating profiles by using multi-parametric analysis, with outputs such as beat rate, peak frequency and width, and waveform irregularities. In addition, the impact of drug treatment on cell viability and mitochondrial integrity was evaluated by high content imaging. To further evaluate the impact of 3D culture on cardiomyocyte responses, we compared the effects (EC50 values) of different compounds in 3D versus 2D culture formats and demonstrated significant differences in assay sensitivity to compound-induced effects. In conclusion, we demonstrated that 3D cardiac spheroids formed with human iPSC-derived cardiomyocytes can be used for drug development and toxicity assessment.

W-1185

ORGANOID CULTURE OF L-MYC IMMORTALIZED HUMAN NEURAL STEM CELLS

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Differentiation of progenitor cells in three dimensional cultures to form self-assembling structures termed organoids has emerged as a valuable technique in basic biology and fundamental/translational medical science. The utility of organiods derives from the extent to which they serve as simulacra of their intended organs along with their accessibility to genetic and environmental manipulations. Here we have used L-myc immortalized neural stem cells (NSCs) to initiate organoid cultures in Matrigel. Culture techniques for LM-NSC008 NSCs are adapted from those previously published protocols for growth of brain organoids. Immunochemical techniques employ extensive permeabilization and overnight exposures to antibody and other reagents to ensure deep penetration, and confocal fluorescence imaging with reconstruction from serial optical sections. Organoids survive without signs of necrosis for over two months. We are presently exploring temporal and spatial expression of differentiation markers including vimentin, nestin, GFAP, tubulin type 3 (Tuj1) doublecortin (DCX) and MAP2 to determine the extent to which these organoids are recapitulating molecular and structural patterns of human cortical development.

Funding Source: Supported by the Beckman Research Institute and the California Institute for Regenerative Medicine Bridges to Stem Cell Research program.

W-1187

NEURAL RETINA DIFFERENTIATION OF HESCS AS AN IN VITRO MODEL FOR RETINOBLASTOMA

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Retinoblastoma is the most common eye tumor of early childhood. Inactivation of both alleles of the retinoblastoma gene (RB1) results in the development of retinoblastoma. Our aim is to establish a human cell-based model for retinoblastoma. Using the CRISPR/Cas9 system we have generated human embryonic stem cells (hESCs) carrying a mutation either on one or both RB1 alleles. All detected mutations are located in exon 3 of

the RB1 gene close to the splice donor site. Analyses on DNA, RNA and protein level were performed for three mutant and one double-mutant clone. The following genotypes were identified by deep sequencing (NM_000321.2): clone C2, c.364_380del, heterozygous; clones C7 and G3, c.372_378del, heterozygous; clone G4, c.[372_378del; c.367_368dup], homozygous (loss of heterozygosity). The mutations of all four clones result in a premature stop codon in exon 4. On RNA level we detected expression of mutant RB1 transcripts reflecting the genotype in all clones and an additional mutant RB1 transcript with skipping of exon 3 in three clones. As the heterozygous clones also showed expression of the wildtype RB1 transcript, RB1 protein (pRb) could be detected for these clones (C2, C7, G3) by western blot analysis. However, the double-mutant clone G4 showed no expression of pRb. So far, we have characterized 3 heterozygous and one homozygous clone. Another three double-mutant clones are under investigation. It has been shown that in 3D culture hESCs can be differentiated into neural retina containing organoids. We established this differentiation schedule and started comparative differentiation of wildtype H1 hESCs and the RB1 null derivative (G4, RB1mt/mt) into neural retina. During the first weeks of differentiation into neural retina organoids generated from the RB1mt/mt hESCs have a smaller diameter and thinner retina layer compared to wildtype organoids. However, during the time-course the mutant organoids began to catch up. Thus, at later stages no difference in size and thickness could be observed anymore. Comparative immunostainings of cryosections at d19 show no difference in expression of the markers PAX6 and SOX2 between the wildtype and mutant hESCs. Further comparative immunostainings for markers specific for neural retina like e.g. RX and VSX2 at d19 and d33 are ongoing and will be presented.

W-1189

CO-SEEDING OF CARDIOMYOCYTES AND PRO-EPICARDIAL CELLS FROM HUMAN IPS CELLS ENHANCES CARDIAC ORGANOID COMPLEXITY

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In cardiac development, the proepicardial organ originates from the anterior surface of the septum transversum. It envelopes the heart to form the epicardium, and plays a critical role in cardiac maturation contributing to cardiomyocyte proliferation, myocardial compaction, and expansion of the coronary plexus. The absence of the proepicardial organ or epicardium results in underdeveloped ventricles, ultimately leading to embryo lethality. To this end, strategies combining proepicardial cells (PECs) with cardiomyocytes (CMs) to engineer the maturation of cardiac tissues ex vivo has not been studied. Previously, we generated human iPS-derived CMs (85.6 ± 8.5% cTnT+) using a Wnt-mediated differentiation protocol. In resulting CM-cultures, we also observed 8.8 ± 3.3% of the supporting non-CM cells expressed the proepicardial marker WT1 by flow cytometry. To enrich this subpopulation, we first enhanced differentiation to lateral plate mesoderm using CHIR99021 for 48 hrs, and then treated with BMP4, VEGF, and retinoic acid for 96 hrs to increase WT1+ expression to 86.8 ± 7.1% within 7 days. Further characterization suggested a definitive proepicardial cell (PEC) population, with upregulated proepicardial genes (WT1, TBX18, TCF21, BNC) and proteins by IF (WT1, ZO1, Tcf21), as well as downregulated cardiac genes (TNNT1, Nkx2.5) and proteins (cTnT). We then differentiated GFP-labeled CMs and mCherry-labeled PECs independently, and recombined the two cell types for co-culture in non-adherent plates. CMs and PECs integrated to form cell aggregates within 24 hrs, and generated spontaneously beating cardiac structures within 72 hrs. Calcium signal imaging at day 7 showed CM/PEC aggregates had shorter action potential durations compared to CM aggregates. CM/PEC aggregates also demonstrated action potential propagation across formed cardiac structures. Immunohistological analysis at day 15 revealed GFP-CMs stained positive for cardiac markers (MHC, cTnT, α-Actinin). Conversely, mCherry-PECs generated WT1+ cells that surrounded aggregates, as well as E-cadherin+, smooth muscle actin+, and calponin+ cells that formed vessel structures within cardiac structures. Taken together, this data suggests CM/PEC co-seeded structures may enhance the ex vivo morphogenesis of engineered myocardial constructs.

W-1191

A NOVEL HIGH-THROUGHPUT PERSONALIZED MEDICINE PLATFORM USING PANCREATIC DUCTAL ADENOCARCINOMA DERIVED ORGANOIDS IN THE ORGANOPLATE®

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Pancreatic cancer is one of the most deadly tumors due to the limited treatment options and late diagnosis. Here, we describe a novel high throughput personalized medicine platform combining the OrganoPlate®, a microfluidic based 3D-culture plate 1, and the recently described Pancreatic Ductal AdenoCarcinoma

(PDAC) derived organoids 2. The OrganoPlate® is a high throughput 3D cell culture microfluidic platform, enabling a wide range of flow and co-culture conditions (e.g. blood vessels), creating physiologically relevant models with a minimal requirement of cell material. Organoids were derived from human PDAC patients and seeded in the OrganoPlate®. Due to the low amount of material required (2500 cells per chip) and the high amount of replicates on one plate (n=96 on a standard microtiter format plate) this makes the OrganoPlate® an efficient platform for personalized medicine and toxicity assays. The viability of the organoids before and after drug treatment is monitored with standard fluorescent viability assays. In addition, medium and cell samples are screened for the presence of metabolites. Metabolic profiling gives insight in the phenotypic changes cancer cells undergo after treatment. In conclusion, the OrganoPlate® is compatible with human pancreatic PDAC derived organoids. The platform can be used for high throughput personalized medicine assays and toxicity screening. 1. Trietsch, S. J., Israëls, G. D., Joore, J., Hankemeier, T. & Vulto, P. Microfluidic titer plate for stratified 3D cell culture. Lab Chip 13, 3548–54 (2013). 2. Boj, S. F. et al. Organoid models of human and mouse ductal pancreatic cancer. Cell 160, 324–338 (2015).

W-1193

TRASNPLANTED HUMAN LUNG ORGNAOIDS DERIVED FROM PLURIPOTENT STEM CELLS REQUIRE A SPECIFIC ENVIRONMENT IN ORDER TO MATURE

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Tissues derived from human pluripotent stem cell (hPSC) often represent early developmental time points. Yet, when transplanted into immunocompromised mice, these derived tissue further mature gaining tissue structure and adult cell types. Previously we have demonstrated that hPSC-derived human lung organoids (HLOs) resembled human fetal lung tissue in vitro (Dye et al. 2015), but when transplanted onto a porous Poly(lactide-co-glycolide) (PLG) scaffold in vivo, the organoids formed tube-like structures that resembled both the structure and cellular diversity of an adult lung airway (Dye et al. 2016). Our initial hypothesis was that the HLOs required a surface such as a microporous scaffold in order for the lung epithelium to survive and mature. However, we have determined that the type of the material that comprises the scaffold is essential for organoids to survive and mature into airway-like structures. Our goal is to define the microenvironment including the material properties of the scaffold, vascular network, and immune response necessary for the transplanted HLOs to mature into adult lung structures.

TISSUE ENGINEERING

W-1197

ADHESION AND VIABILITY OF MESENCHYMAL CELLS OF DIFFERENT SOURCES ON ALIGNED POLY(LACTIC-CO-GLYCOLIC ACID) FIBROUS SCAFFOLDS

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Mesenchymal stem cells (MSCs) are a promising tool for cell therapy through their ability to reduce inflammation and differentiating into different lineages, including neural cells. Peripheral nerve damage affects thousands of people yearly worldwide. Tissue engineering products offer an alternative to the traditional autologous nerve grafts used to treat nerve defects. Conduits made of aligned nanofibers and MSCs may contribute to nerve regeneration by providing a platform for oriented nerve fiber growth and may be an appropriate source of cells for regeneration. The aim of this study has been to analyze the cytocompatibility of mesenchymal stem cells derived from human exfoliated deciduous teeth and umbilical cord cultured onto conduits of aligned PLGA fibers. An 18% poly lactic-co-glycolic acid (PLGA) solution in hexafluor-2-propanol was used to produce a scaffold of aligned nanofibers. A conduit with 1.5 mm diameter and 5 mm length was produced by rolling the aligned nanofibers around a needle and fixing the edges with the polymer solution. MSCs from the two different sources were seeded on the conduits, either only interior, exterior or on both surfaces. The conduits were cultivated for 1 and 7 days to evaluate cell adhesion, migration and viability by confocal microscopy, live/dead and WST8 assays. Statistical analyses were performed by the one-way ANOVA test. For evaluation of adhesion, the cells were stained with DAPI/rhodamine-phalloidin and analyzed by confocal microscopy. The MSCs from the two different sources showed good adhesion and spread onto the conduit, with more cells adhering into the lumen of the conduit. Cell viability was assessed by live/dead and WST-8 assays and showed that the adhered cells of the three groups (cells inside the conduit, cells outside and cells inside and outside the conduit) maintained viable on the biomaterial. Despite the MSCs from the deciduous teeth having shown better cell adhesion and viability rate in comparison with the MCSs from the umbilical cord , both sources of cells showed good results in these biological analyses.

In conclusion, the MSCs from both sources adhered and maintained viability on the conduits, showing that the conduit has good biocompatibility and can be used as a regenerative product in peripheral nerve injury.

Funding Source: CNPq, FINEP, MCTI, and Stem Cell Research Institute

W-1199

OSTEOGENIC PROGRAMMING OF HUMAN MESENCHYMAL STEM CELLS WITH HIGHLY EFFICIENT INTRACELLULAR DELIVERY OF RUNX2

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Mesenchymal stem cells (MSCs) have versatile regenerative potential due to their tri-lineage differentiation and immunomodulation activity. Currently there are two major challenges in directed differentiation of MSCs for therapeutic applications. Firstly, MSCs are difficult to transfect with existing approaches which are clinically unattractive (viral transfection) or have low efficacy (lipid-mediated transfection). Secondly, chemical and growth factor strategies to direct osteogenesis in vivo, lack specificity for targeted delivery with desired effects. To overcome these challenges we delivered recombinant transcription factors (TFs) with the GAG-binding enhanced transduction (GET) delivery system (fusion of P21 and 8R peptides). We used the osteogenic master reguzlator, RUNX2 as a programming factor due to its stage-specific role in osteochondral differentiation pathways. We engineered GET-fusion proteins and compared sequential osteogenic changes in MSCs, induced by exposure to GET fusion proteins or conventional stimulation methods (Dexamethasone and BMP2). By assessing loss of stem cell-surface markers, upregulation of osteogenic genes and matrix mineralization, herein we demonstrate that GET-RUNX2 effectively transduces MSCs and triggers osteogenesis by enhancing target gene expression. The high transduction efficiency of GET-system holds great potential in stem cell therapies by allowing direct transcriptional control over stem cells, bypassing problems observed with high-concentration growth-factor or steroid therapies.

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W-1201

PROTEINS EXTRACTED FROM HUMAN BONE MARROW ENHANCE MESENCHYMAL STEM CELL ACTIVITIES

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Maintaining properties of human bone marrow-derived mesenchymal stem cells (BMSCs) in culture for regenerative applications remains a great challenge. An emerging approach of constructing a culture environment mimicking the bone marrow niche to regulate BMSC activities has been developed. In this study, we have demonstrated a systematic approach to identify soluble factors of interest extracted from human bone marrow and used them in BMSC culture for tissue regeneration. We have found that lipocalin-2 and prolactin are key factors in bone marrow, involved in regulating BMSC activities. Treating the cell with lipocalin-2 and prolactin delays cellular senescence of BMSCs and primes the cell for osteogenesis and chondrogenesis. We have also demonstrated that BMSCs pretreated with lipocalin-2 and prolactin can enhance the repair of calvarial defects in mice. Together, our study provides research evidence of using a viable approach to prime BMSC properties in vitro for improving cell-based tissue regeneration in vivo.

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W-1203

PANCREATIC ALPHA CELL INDUCTION OF POLYHORMONAL PRECURSORS IN A BIOMIMETIC ISLET CAPSULE

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Human islet research is limited by the inability of conventional culture conditions to maintain the functionality of cadavers or to mature islet organoids derived from human pluripotent stem cells. Recapitulation of the islet-specific microenvironment is a promising potential solution, as the physical properties of culture substrates demonstrably influence the structure and function of islet cells. While popular hydrogel substrates have tunable properties and can protect islets from immune responses following transplantation into animals, decreased viability is often observed in hydrogel encapsulated islet cells in culture. Inspired by the native extracellular matrix, we designed

a nanofibrous scaffold mimicking the three-dimensional structure and stiffness of the human islet capsule. Using stem-cell derived islet cells, we tested our force spun scaffold in prolonged culture. After one week, viability of islet cells cultured in the scaffold remained unchanged while a near 50% reduction in viability was observed for hydrogel encapsulated cells. Over a one-month time course, multicolor flow cytometry further revealed an increase in α-cell differentiation in the scaffold via polyhormonal intermediates. We also reconstituted the protein composition of the human islet extracellular matrix on our scaffold and are now determining the extent to which biomimicry of both the physical and biochemical islet microenvironment can mature gene expression profiles and glucose stimulated insulin secretion in stem cell derived islet cells in vitro.

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W-1205

ENGRAFTMENT AND FUNCTION OF HUMAN PLURIPOTENT STEM CELL-DERIVED HEPATOCYTE-LIKE CELLS IN MICE VIA 3D CO-AGGREGATION AND ENCAPSULATION

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Cellular therapies for liver diseases and in vitro models for drug testing both require functional human hepatocytes, which have unfortunately been limited due to the paucity of donor liver tissues. Human pluripotent stem cells represent a promising and potentially unlimited cell source to derive human hepatocytes. However, the hepatic functions of these human pluripotent stem cells-derived cells to date are not fully comparable to adult human hepatocytes and are more similar to fetal ones. In addition, it has been challenging to obtain functional hepatic engraftment of these cells with prior studies having been done inimmunocompromised animals. In this report, we demonstrated successful engraftment of human induced pluripotent stem cell derived hepatocyte-like cells in immunocompetent mice by pre-engineering 3D cell co-aggregates with stromal cells followed by encapsulation in recently developed biocompatible hydrogel capsules. Notably, upon transplantation, human albumin and α1-antitrypsin (A1AT) in mouse sera secreted by encapsulated induced pluripotent stem cell derived hepatocyte-like cells/stromal cell aggregates reached a level comparable to the primary human hepatocyte/stromal cell control. Further immunohistochemistry of human albumin in retrieved cell aggregates confirmed the survival and function of iPS-H. This proof-of-concept study provides a simple yet robust approach to improve the engraftment of induced pluripotent stem cell derived hepatocyte-like cells, and may be applicable to many stem cell-based therapies.

W-1207

ZONAL 3D PRINTING OF CELL-LADEN GENE ACTIVATED BIOINKS FOR INTERFACE TISSUE ENGINEERING

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Engineering complex interface tissues such as the osteochondral unit requires precise spatial control of cellular differentiation and matrix deposition and organization. Additive fabrication techniques such as 3D bioprinting can be used to spatially deposit different materials (bioinks) and bioactive molecules. The incorporation of gene delivery into a bioink offers a unique approach for the controlled release of therapeutic factors in a spatially and temporally defined manner in order to control the differentiation of bioprinted mesenchymal stem cells (MSCs). The objective of this study was to develop a chondro-inductive and an osteo-inductive gene activated bioink (GAB) for the 3D printing of an osteochondral construct. For this purpose, two different MSC-laden GABs were developed: (1) a chondro-inductive GAB consisting of the incorporation of MSCs and RALA peptide-plasmid DNA (pDNA) complexes into unmodified alginate, and (2) an osteo-inductive GAB consisting of the incorporation of MSCs and nanohydroxyapatite (nHA)-pDNA complexes into a RGD-modified alginate hydrogel. Green fluorescent protein (GFP) and luciferase analysis showed effective transfection over time of the encapsulated MSCs. Co-printing of GFP and red fluorescent protein (RFP) transfected cells demonstrated precise spatial control of protein deposition inside the construct, confirming zonal MSC transfection. Next, bi-layered constructs were printed by the serial deposition of chondro-inductive (pTGF-β3 and pBMP2) and osteo-inductive (pBMP2) bioinks. After 21 days of in vitro culture, significantly higher levels of mineralization were observed in the osteo layer, while significantly higher levels of cartilage specific extracellular matrix accumulation were observed in the chondro layer, reproducing the biochemical gradients found in osteochondral tissue. This study highlights the potential of the 3D bioprinting of gene activated materials for the spatial delivery of genes for

the local production of growth factors, thereby enabling the recapitulation of the biochemical and phenotypical gradients found in native tissues.

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W-1209

SUBSTANCE-P STIMULATED MOBILIZATION OF BOTH EPC AND BMSC FROM THE BONE MARROW TO THE BLOOD FOR ENGAGEMENT IN THE HEALING TISSUE OF THE ALKALI BURN RABBIT EYE

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The bone marrow (BM) is a source of stem cells such as hematopoietic stem cells, endothelial precursor cells (EPC), and bone marrow stromal cell (BMSC, also generally called as mesenchymal stem cell), which can be mobilized to damaged tissues for repair. However, molecules and mechanisms underlying mobilization and homing of these cells to target tissues are not clearly defined. Here, we report that substance-P (SP), a known neuropeptide, stimulated the proliferation of both EPC and BMSC in the bone marrow and their mobilization to the peripheral blood via intra-bone marrow injection of SP and BrdU incorporation assay. This function of SP could be specifically blocked by NK-1 Receptor antagonist and compared with GCSF, which could mobilize EPC only. SP-mobilized EPC and BMSC harvested from the blood were concertedly engaged in the neo-vasculogenesis as endothelial cells and alpha-smooth muscle actin-expressing pericytes, respectively, in the nude mice matrigel plug assay. SP injection itself markedly increased the pericyte coverage on the endothelia of the healing tissue in the alkali-burn eye injury. Furthermore, PKH-26 labeled SP-mobilized EPC and BMSC were engaged in the pericyte-covered endothelia in the alkali burn eye in contrast to much less pericyte coverage on the enlarged endothelia with EPC only transfusion. This new function of SP may substitute elaborate ex vivo cell culture of therapeutic cells through its stimulation of stem cell proliferation in the bone marrow in vivo and mobilization of those therapeutic cells to the patient own blood stream for the tissue repair such as myocardiac infarction, limb ischemia, and stroke, whose example will be presented.

Funding Source: This study was supported by Korean Health Technology R&D Project grant (HI13C1479) from the Ministry of Health and Welfare and Bio & Medical Technology Development Program (NRF-2012M3A9C6050499) of the National Research Foundation.

W-1211

RHOA/ROCK SIGNALING DURING THE DEVELOPMENT OF HUMAN MSC-BASED ENGINEERED CHONDROGENIC TISSUE

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Chondrogenesis can be induced in vitro in hMSCs by certain bioactive factors such as transforming growth factor-β, but the biochemical and biomechanical properties of the resulting cartilage constructs are inferior compared to native tissue. The goal of this study is to develop strategies to bridge this gap in tissue constructs. RhoA/ROCK signaling drives commitment of hMSCs between osteoblast and adipocyte fate via regulating actomyosin contractility, but its role in hMSC chondrogenesis has not been studied. The actomyosin contractility is inferred to be involved in hMSC rounding and aggregation at the onset of chondrogenesis. Therefore, we hypothesize that inhibition of RhoA/ROCK signaling will lead to reduced cytoskeletal tension in chondrogenic hMSCs and thereby forming less dense aggregates with enhanced transport properties. The resulting ROCK-inhibited hMSC aggregates supported our hypothesis evidenced by down-regulated contraction force (28% reduced) using traction force microscopy technique and significantly increased construct size (198%). The larger aggregates had lower cell density, and its cellularity was closer to native adult articular cartilage. The increased space between cells within the aggregate led to overall enhanced transport proportional to the size illustrated by the fluorescent-labeled dextran diffusion and glucose uptake profiles. In addition, the increase in water content upon ROCK inhibition could also account for the diffusional enhancement. The key cartilaginous matrix, glycosaminoglycans (GAG) and collagen, were also significantly enhanced by 33% and 18% respectively to fill the increased interstitial space between cells. The increased glucose consumption upon ROCK inhibition was, therefore, likely due to its enhanced transport and was positively related to the enhanced synthesis of GAG and collagen. Our findings indicate that repression of cytoskeletal tension thus regulating spontaneous cell rounding and contraction during early differentiation could be important to achieve improved microenviroment for hMSC-derived cartilage culture. We expect that these findings will be ultimately applied to facilitate cartilaginous tissue culture from hMSCs.

Funding Source: The above work is funded in part by a grant from the National Institutes of Health (EB021911)

W-1213

ELIMINATION OF RESIDUAL IPS CELLS IN IPS-DERIVED CARDIAC TISSUE BY CDKS INHIBITION

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Induced Pluripotent Stem Cells (iPSCs) hold great potential for being a major source of cells for regenerative medicine. Since their discovery, human iPSCs were successfully differentiated to numerous functional cell types that closely resembled those found in primary human tissue. However, one of the remaining issues that need to be resolved before the full potential of iPSCs can be seen in clinic, is the risk of teratoma formation. The presence of undifferentiated cells in iPS-derived tissue may cause tumor formation in patients following transplantation. Therefore there is a pressing need to develop highly efficient techniques that can completely eliminate remaining iPSCs. The need for such techniques substantially increases when treating major organs like the heart, where a minimum of one billion of iPS-derived cardiac cells are required. In this report we show that the CDKs inhibitor, Dinaciclib, selectively eliminates iPS cells without affecting the viability of cardiac cells. We found that low nanomolar concentrations of dinaciclib were enough to induce cell death, increase DNA damage and p53 protein levels in human iPS cells. This was accompanied by negative regulation of the anti-apoptotic protein MCL-1. Gene knockdown experiments revealed that p53 downregulation only increases the threshold of dinaciclib induced apoptosis in iPS cells but does not inhibit it. Dinaciclib inhibited CDK9 activity and the phosphorylation of Serine 2 of the C-terminal domain of RNA Polyemrase II. This resulted in the inhibition of transcription of MCL-1 and the pluripotency genes, NANOG and c-MYC. Even though dinaciclib caused a slight downregulation of MCL-1 in iPS-derived cardiac cells, the viability of the cells was not significantly affected, and beating iPS-derived cardiac cell sheet could still be fabricated. These findings suggest a difference in tolerance of MCL-1 downregulation between iPSCs and iPS-derived cardiac cells which could be exploited to eliminate remaining iPS cells in bioengineered cell sheet tissues.

Funding Source: Projects for Technological Development in Research Center Network for Realization of Regenerative Medicine of the Japan Science and Technology Agency, JST and Japan Agency for Medical Research and Development, AMED.

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

W-1217

MINI-ORGANS IN A DISH: HOW DO CF PATIENTS VIEW ORGANOIDS

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The emerging possibility to culture human tissues in vitro raises considerable expectation among the biomedical community and general public. These organoids promise various scientific and clinical merits. The ability to bank and widely distribute organoids further enhances their potential. Next to its promises, however, organoid technology revives old and raises new ethical challenges, such as questions about ownership, consent, and commercialization. In the emerging ethical debate the perspective of donors on organoid technology is still missing. To what extend do they view the donation of tissue for organoids the same or differently from other types of cell and tissue research? Our study is as far as we know the first empirical study that scrutinizes the views of organoid donors. One promising field where gut organoids of patients enable personalized medicine is Cystic Fibrosis (CF) research and care. In a qualitative interview study, we examined the experiences, opinions, and attitudes of CF patients and their parents. From the 23 in-depth interviews that we conducted, it follows that despite their initial positive attitude towards organoid technology, our respondents raise three clusters of concerns. First, CF patients relate the mini-guts to their (bodily) identity and wellbeing, although in ambivalent ways. Organoids are tangible and immortal entities that can directly inform clinical treatment through personalized drug testing. This personal connection can clash with the technological and commercial potential of organoids. Second, respondents struggle with the open-endedness of organoid banking and use. They wish to compensate this through restricted consent, continuous engagement, and responsible stewardship. Third, respondents are particularly cautious with regard

to commercialization of organoid technology. Concerns they raise relate to injustice, exploitation, incompatibility with altruism, lack of oversight, and fear of adverse consequences. Our findings have important policy implications for the governance of organoid banking. Flexible use of organoids, necessary for optimizing their scientific and clinical merits, should be balanced with individual control. Sound governance entails flexible consent options, adaptive governance structures and fair benefit-sharing.

W-1219

THE ANDALUSIAN INITIATIVE FOR ADVANCED THERAPIES: A TECHNOLOGY MATURATION PLATFORM FOSTERING CLINICAL TRANSLATION OF CELL AND GENE THERAPIES

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Andalusia, with over 700 patients enrolled in academic clinical trials or receiving investigational cell-based therapies, is the most active European Region in the clinical translation of Advanced Therapy Medicinal Products (ATMPs) within a public system. This outstanding result has been made possible partly because of a pioneer organizational model, the Andalusian Initiative for Advanced Therapies (AIAT), created by the Regional Government in 2009 to promote R&D&i activities in the field of Regenerative Medicine. Our mission is to foster research, maturation and translation of cell and gene therapies, and to coordinate the provision of regenerative medicine treatments within the Andalusian Public Healthcare System (APHS). As part of the APHS, provider of health services to 8.5 million people, we work within an impressive network of hospitals, primary care centres, research centres and institutes, and a Biobank storing over 1 million samples, including hiPS and hES cell lines. AIAT built and directly coordinates a network of 10 GMP facilities where gene and cell-based therapies are manufactured under strict regulatory standards. The AIAT fosters national as well as international alliances between academia, biotech, hospitals, SMEs and the pharmaceutical industry. Indeed, the coordination unit of AIAT has acted as promoter in 26 clinical trials involving cell-based therapies and has established more than 50 licencing and public-private partnership agreements. Notwithstanding, we would like to highlight here the importance of education in the success of our program. In addition to ethical, regulatory and scientific advice with the support of the Spanish Medicine Agency, we have put a big effort in the organization of specific training courses -Good Laboratory Practice (GLP), Good Clinical Practice (GCP), Good Distribution Practice of Medicinal Products (GDP)- and, in collaboration with the University of Granada, an International Master degree in ATMP Manufacturing, unique in Europe. Overall, we have trained 1,375 professionals who will certainly contribute to the advancement of this field.

W-1221

NEW GUIDELINES FOR CELL THERAPY AGAINST STROKE ENCOURAGE THE DEVELOPMENT IN JAPAN

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A recent breakthrough in cell therapy is expected to reverse the neurological sequelae of stroke. Although some pioneer studies of cell therapy against stroke have been reported, the failure of them declared that certain problems still remained unsolved. Some researchers in the US intended to formulate guidelines for the development of cell therapy against stroke. In 2007, Stem cell Therapeutics as an Emerging Paradigm in Stroke (STEPS) group, whose members belonged to academia, industry, and National Institutes of Health (NIH), launched an effort to set the standards for the development of cell therapy. The first recommendation, STEPS-I, was published in 2009. It included the design of the pre-clinical studies and the design of the early phase of clinical trials. Since a series of STEPS, many clinical trials concerning cell therapy against stroke have been initiated worldwide. These results show that a series of STEPS has encouraged the development of new cell therapies all over the world. To encourage basic science to be translated into bedside in Japan, Ministry of Health, Labour and Welfare started the new project, "Initiative for Accelerating Regulatory Science in Innovative Drug, Medical Device, and Regenerative Medicine" in 2012. The project promoted to establish various guidelines for the development of new cell-products. As a part of the project, the working group (chairman, Dr. Kiyohiro Houkin, Hokkaido University, Sapporo, Japan) about new guidelines for cell therapy against stroke started in November 2013. It consisted of physicians, basic and regulatory scientists, and Pharmaceuticals and Medical Devices Agency (PMDA) staffs. It was important to show an original stance in Japan because of domestic regulations for regenerative medicine, although a series of STEPS was used as a reference. It was noteworthy that not only the researchers but also PMDA could use the guideline for reviews. In 2016, the guidelines will be launched in Japanese, and then the text will be translated into English to propagate it worldwide. It is believed

that the new guidelines will promote the development of new cell therapies in Japan and will be established for stroke management in the future.

W-1223

DEVELOPING TREATMENTS FOR MITOCHONDRIAL DISEASE WITHIN THE CONFLUENCE OF SCIENCE, ETHICS, AND REGULATION

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Growing understanding of mitochondrial disease has led to efforts now under way to develop treatments based on techniques arising from stem cell science and technology. These efforts have been, and will continue to be, subject to the confluence of stem cell science, ethics, and regulatory compliance. Recent U.S. legislation (most notably, the signing into law of the 21st Century Cures Act) and the transition in the U.S. executive branch are likely to have an impact on this confluence. The 21st Century Cures Act, for example, contains provisions intended to spur the development of regenerative advanced therapies for serious and life-threatening diseases through application of the U.S. Food and Drug Administration's expedited approval pathways, and contemplates the promulgation by FDA and other agencies of applicable regulations, guidances, and standards for that purpose. At the same time, the new administration is seeking to manage the growth of regulations within the executive branch. Against this backdrop, at least three questions arise: (1) generally, will NIH propose and adopt any changes in its current Guidelines on Human Stem Cell Research? (2) more particularly, will pending amendments to the NIH Guidelines regarding chimera research be adopted or abandoned? (3) what will be the impact of the answers to the first two questions on therapies under development for treating mitochondrial disease? We will explore each of these three questions.

CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

W-1225

EFFICIENT GENERATION OF CHEMICALLY INDUCED MESENCHYMAL STEM CELLS FROM HUMAN DERMAL FIBROBLASTS

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Due to the multipotency, immune-modularity, and the safety concern without tumorigenicity, human mesenchymal stromal/stem cells (MSCs) hold great promise in regenerative medicine and cell therapies. To date, MSCs already approved to treat Graft-versus-host disease, degenerative arthritis, and anal fistula in human and undergoing 685 clinical trials for the treatment of at least 10 different kinds of disease. To avoid (1) insertional mutagenesis by virus infection or plasmid transfection, (2) the tedious processes of virus preparation, and (3) repeated transfection/transduction, the use of chemical/growth factors to convert fibroblasts to functional cells has drawn substantial attention recently. However, no previous study has generated induced MSC (iMSCs) from skin fibroblasts with chemicals and/or growth factors. Herein, we established the first method to generate functional iMSCs from primary human dermal fibroblasts by solely small molecules with or without growth factors. The protocol can enrich iMSCs in only 6 days with an average conversion rate of 38%. Like traditional stem cells, only iMSCs, but not fibroblasts have clonogenicity. In addition, our microarray displayed that iMSCs generated from one neonatal and two adult fibroblasts are more similar to bone marrow MSCs (BMMSCs) while compared to their parental fibroblasts. The phenotype of iMSCs fulfills all of the criteria of traditional MSCs as determined by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT). Most of all, the iMSCs can be further differentiated into osteoblasts, adipocytes, and chondrocytes to a degree comparable to BMMSCs. The chemicals can be removed, and iMSCs can expand in regular culture medium up to 8 passages. Of note, the iMSCs can suppress endotoxin

(lipopolysaccharide, LPS)-mediated acute lung injury as effectively as BMMSCs by completely recuse the lethality and decrease injury score. We found 3 chemicals are essential for producing iMSCs while 6 chemicals have the best efficiency. Overall, in this study, we reveal a brand new strategy and breakthrough protocol to generate iMSCs from skin fibroblasts that mimic BMMSCs. Thus, iMSCs can be an easily accessible resource to enrich sufficient BMMSC like cells for research in cell biology and regenerative medicine.

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W-1227

INDUCTION OF OLIGODENDROCYTE PROGENITOR CELLS AND FUNCTIONAL OLIGODENDROCYTES FROM IPSCS WITH MRNA FOR GMP MANUFACTURING AND CELL THERAP

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Here we report a newly developed protocols, which direct iPSCs differentiation to oligodendrocyte progenitor cells (OPCs) and functional oligodendrocytes. Oligodendrocyte lineage specific transcription factors were introduced into iPSCs to direct the differentiation via in vitro transcribed mRNA. In 2 weeks, typical OPCs morphology appeared. These cells were ^100% homogenous, and were identified by NG2+, A2B5, O4 and PDGF R Alpha staining. These cells can be further cultured in maturation medium in either suspension or attached culture system. Mature oligodendrocytes morphology appeared in 6 weeks, and were identified by myelin-related proteins, such as MBP, MAG, MOG, OMgp, and CNPase. Co-culture system of Oligodendrocyte and Moto-neuron has been further developed. The proliferation, migration, differentiation and membrane wrapping will be observed for functional analysis. Animal studies that aims at spinal injury therapy have been planned and results will be presented. These protocols will be adapted into Allele Biotech's GMP Stem Cell Manufacture system with full cGMP compliance, and shall be ready for any GLP safety evaluation and clinical studies.

W-1229

HIGH EXPRESSION OF SLC35F2 IN HUMAN PLURIPOTENT STEM CELLS ENABLES YM155 MEDIATED SELECTIVE CELL DEATH

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Risk of teratoma formation has been considered to one of the most serious technical hurdles of human pluripotent stem cells (hPSCs) based cell therapy. To resolve this, a variety of approaches including small molecules have been developed to selectively ablate the residual undifferentiated hPSCs. Previously, we reported that treatment of YM155, a Survivin inhibitor, selectively eliminates hPSCs by inducing p53 mitochondrial translocation and following apoptosis. However, it remains still unclear how high sensitivity of YM155 in hPSCs but not differentiated cells is determined. Herein, we demonstrate that YM155 induces selective DNA damage in hPSCs, activating 'high mitochondrial priming'. By screening drug transporters, located in plasma membrane, we found that a solute carrier family member 35 F2 (SLC35F2), which was previously reported to mediate YM155 intake in cancer cells were highly expressed in hPSCs but not in differentiated counterpart. By establishing SLC35F2 knockout (KO) hPSCs, which were resistant to YM155 treatment, we conclude that high expression of SLC35F2 in hPSCs is responsible for YM155 mediated cell death.

W-1231

HIGHLY EFFICIENT RNA BASED REPROGRAMMING AND GENE EDITING FOR THE MODELING AND TREATMENT OF SKIN BLISTERING DISEASES

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Coupling the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) with targeted gene correction using CRISPR/Cas9 offers the possibility of developing a new stem cell-based approach for the treatment of inherited skin blistering diseases such as Epidermolysis Bullosa (EB). Many protocols for reprogramming and gene correction depend on viral- or plasmid-based expression, which are not ideal for clinical applications due to the risk of insertional mutagenesis. A more clinically suitable approach should rely on footprint-free reprogramming and genome modifications. Toward this goal, we have developed a novel integration- and feeder-free RNA-based

approach for the reprogramming of patient-derived cells into iPSCs with unprecedented efficiency. Using this methodology, we have successfully generated iPSCs from fibroblasts isolated from patients with EB simplex (heterozygous KRT14, 125R>C) and recessive dystrophic EB (homozygous COL7A1, c.7485+5G>A). To correct these mutations, we designed two plasmids for T7-based in vitro synthesis of Cas9 modified mRNA and guide RNA. The Cas9 expression plasmid also encodes the transcript stabilizing 3'UTR from human β-Globin followed by an 85-bp poly(A) tail. Co-transfection of these two components efficiently induces double strand breaks (>30% without positive selection), assessed by a T7E1 assay for INDEL formation. Furthermore, we have identified a guide RNA that can differentiate the single base pair difference between mutant and wild type alleles, thereby selectively cutting only the desired mutant allele. This is an important design consideration, as it is critical to avoid off-target mutagenesis in paralogous genes containing extremely high homology to KRT14. Additionally, selectively cutting mutant but not corrected wild-type DNA should enhance repair efficiencies without the need for introduction of "scarring" silent mutations. We aim to combine these approaches for simultaneous reprogramming and gene correction, thereby avoiding lengthy cell culture periods, drug selection, and multiple sub-cloning steps.

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

W-2001

PDGFRA + CELLS IN MOUSE EMBRYONIC STEM CELL CULTURES REPRESENT THE IN VITRO EQUIVALENT OF THE PRE-IMPLANTATION PRIMITIVE ENDODERM PRECURSORS

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In early mouse pre-implantation development, primitive endoderm (PrE) precursors are platelet-derived growth factor receptor alpha (PDGFRa) positive. Here, we demonstrated that cultured mouse embryonic stem cells (mESCs) express PDGFRa heterogeneously, fluctuating between a PDGFRa+ (PrE-primed) and a platelet endothelial cell adhesion molecule 1 (PECAM1)-positive state (epiblast-primed). The two surface markers can be co-detected on a third subpopulation, expressing epiblast and PrE determinants (double-positive). In vitro, these subpopulations differ in their self-renewal and differentiation capability, transcriptional and epigenetic states. In vivo, double-positive cells contributed to epiblast and PrE, while PrE-primed cells exclusively contributed to PrE derivatives. The transcriptome of PDGFRa+ subpopulations differs from previously described subpopulations and shows similarities with early/mid blastocyst cells. The heterogeneity did not depend on PDGFRa but on leukemia inhibitory factor and fibroblast growth factor signaling and DNA methylation. Thus, PDGFRa+ cells represent the in vitro counterpart of in vivo PrE precursors, and their selection from cultured mESCs can yield pure PrE precursors.

W-2003

LOSS OF P66SHC ACCELERATES THE CELL CYCLE AND THE ONSET OF GATA4 EXPRESSION IN MOUSE BLASTOCYSTS

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During oxidative stress, the adaptor protein p66Shc produces mitochondrial reactive oxygen species that trigger apoptosis and cell cycle arrest. However, p66Shc is also known to regulate proliferation and metabolism through MAPK and mTOR-dependent signaling. Due to its varying functions in different cell types, it is not clear what the biological role of p66Shc is during preimplantation embryo development. We have recently discovered that p66Shc expression is upregulated in mouse blastocysts and p66Shc protein is detectable in the trophectoderm. Thus, the aim of this study is to determine the function(s) of p66Shc in regulating mouse blastocyst development. Here we show that a reduced p66Shc transcript abundance after its siRNA-mediated knockdown in mouse zygotes accelerates blastocyst development in vitro. Embryos with reduced p66Shc formed blastocysts containing significantly more cells than controls. Increased cell number in p66Shc knockdown blastocysts is due to increased cell proliferation, as p66Shc knockdown blastocysts contained more BrdU positive cells. In addition, p66Shc knockdown blastocysts had significantly decreased transcript abundance of retinoblastoma 1, a G1/S checkpoint marker, compared to controls. Blastocyst lineage marker expression was altered in p66Shc knockdown embryos. Sixty percent of p66Shc knockdown blastocysts showed earlier (E3.5) OCT3/4 restriction to the inner cell mass compared to 25% of control blastocysts. Furthermore, p66Shc knockdown

embryos contained a higher percentage of cells positive for the primitive endoderm marker GATA4. At E4.5, 36% of p66Shc knockdown blastocysts contained GATA4 positive cells sorted to the primitive endoderm layer, compared to 14% of the control blastocyst population containing sorted GATA4 cells. Our results suggest that loss of p66Shc accelerates the embryonic cell cycle and induces precocious specification of the primitive endoderm. Therefore, in addition to its role during the oxidative stress response, we predict that p66Shc may act to regulate blastomere proliferation, which then establishes normal timing for lineage commitment in the blastocyst.

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PLURIPOTENCY

W-2005

VITAMIN C DERIVATIVE STABLY INDUCES NAÏVE STATE OF MURINE EMBRYONIC STEM CELL IN A TET DEPENDENT MANNER

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Response to culture environment, pluripotent stem cells (PSCs) change the epigenetic status, particularly DNA methylation and the resulting gene expression program could determine the developmental status of PSCs. It is widely known that vitamin C (VitC) is beneficial to induce a naïve status or enhance somatic cell reprogramming through various epigenetic mechanisms. However, VitC is unstable because of structural problems, also toxicity within the cell. Here, we show that a VitC stabilized derivate, ascorbic acid 2-glucoside (AA2G) overcomes the limits of VitC and more efficiently induced the naïve status of murine embryonic stem cells (mESCs) and established the inducible pluripotent stem cells (iPSCs). Unlike VitC, AA2G was very stable more than 1 month in culture medium and increased the proliferation of mESCs with little cellular toxicity even in high dosage. Cultivation of mESCs under supplement of AA2G increased the naïve stated cells than normal FBS-containing medium, evidenced by the increase in alkaline phosphatase activity, gene expression profiling, and distal enhancer activity of Oct4 promoter. Mechanistically, AA2G supplement to mESCs, as similar to VitC, promotes ten eleven translocation (Tet) dependent DNA demethylation and following upregulation of demethylated germline genes which were significantly impaired in Tet1 and Tet2 double knockout ESCs. Furthermore, AA2G enhances the reprogramming process of murine somatic cells. Taken together, the present study demonstrate that culture of PSCs with AA2G supplement provide a stable environment favorable to naïve pluripotent state through a distinct epigenetic mechanism that could be beneficial to enforce epigenetic stability and developmental potency of PSCs.

W-2007

ASSAY DEVELOPMENT AND CELLL CHARACTERIZATION CHALLENGES OF HUMAN INDUCED PLURIPOTENT STEM CELLS FOR CELL THERAPY APPLICATIONS

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Human induced pluripotent stem cells (iPSC) hold tremendous potentials for use in cell therapy applications. To develop clinical quantities of functional iPSC derived products under current good manufacturing practices (cGMP), it is important to develop characterization assays to evaluate the critical quality attributes of these cells. However, assay development is facing several challenges including (1) establishing relevant characterization platform to meet the quality requirements of these cells, (2) comparability or equivalency of the products developed from iPSCs that are intrinsically variable and can change during the manufacturing process and/or after transplantation in response to the environment, and (3) development of sensitive assays that can quantitatively measure cellular characteristics in particular the safety and functionality of iPSC derived products. To address these challenges, we first established a characterization platform by classifying the assays into two main groups: (1) release assays that require optimization and qualification of the tests, and (2) For Information Only (FIO) or characterization assays used to collect more information about the final product. We implemented new biological assays such as whole genome sequencing (WGS), array-based analysis, and comparative genomic hybridization (aCGH) single nucleotide polymorphism (SNP) analysis in our characterization platform. Our goal was to provide data to the end users to determine which subset of tests will be required for on-going

monitoring, how such tests should be used to evaluate the use of iPSC subclones for preclinical studies or cell therapy, and how comparability between manufacturing sites needs to be established. We have been able to use this characterization platform along with the new biological assays for detailed characterization of two human iPSC clones manufactured under cGMP. We have demonstrated that these new assays can provide comprehensive information to overcome the current limitations with the sensitivity of analytical methods and can serve as an unlimited source of information to determine the biological utility of the final cell therapy products.

W-2009

GENERATION OF COMPLETE-CHEMICAL INDUCED PLURIPOTENT STEM CELLS FROM ADULT GRANULOSA CELLS

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Pluripotent stem cells can be induced from mouse somatic cells by chemical approach, and full-chemical induced pluripotent stem cells (CiPSCs) may have great potential in clinical applications. However, successful induction of CiPSCs has been limited to few cell types and remains inefficient. Granulosa cells residing in the ovarian follicles possess characteristics of stem cells, as evidenced by expression of Klf4 and c-Myc and by minimal level of nuclear membrane LaminA, such that they can be reprogrammed into iPS cells with fewer transcription factors. Moreover, granulosa cells and cumulus cells surrounding oocytes are readily accessible and as by-products are often discarded from in vitro fertilization (IVF) clinic. Here we show that CiPSCs can be effectively generated from granulosa cells using a three-step method (Zhao et al. Cell 2015), and notably by activation of Zscan4, a 2-cell embryo gene that is sporadically expressed in a ES cell population and involved in telomere lengthening and genomic stability. In contrast, CiPSC clones were not formed without activation of Zscan4 during reprogramming induction, suggesting that Zscan4 expression plays an important role in the full chemical reprogramming of granulosa cells. Granulosa cells were isolated from ovarian follicles of Oct4-DE-EGFP mice and 1~2% Oct4-DE-EGFP positive cells were obtained by 30 days of reprogramming induction, earlier than the induction from mouse embryonic fibroblasts served as a control. The CiPSCs induced from granulosa cells expressed high levels of genes associated with pluripotency, and can successfully differentiate into three embryonic germ layers after differentiation induction in vitro, and also in vivo by teratoma formation test after injection into NOD-SCID mice. We will report the chimera production test and germline transmission experiment data. Together, these results demonstrate that full chemical reprogramming approach can be optimized to accelerate and generate CiPSCs from granulosa cells, the definitive female adult somatic cells.

W-2011

CDK11 SUPERVISES THE ACTIVATION OF FGFR SIGNALING TO SUSTAIN HESC SELF-RENEWAL AND PLURIPOTENCY

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Human embryonic stem cells (hESCs) have two unique characteristics, unlimited self-renewal and pluripotency. Among the factors that control the properties of hESCs, extracellular signals and intracellular core transcriptional factors have been extensively investigated. However, how extracellular signals connect to the transcriptional circuitry remains poorly understood. To address this question, we carry out a genome-wide siRNA screening to find important protein kinases in hESCs, as protein kinases are critical factors downstream of extracellular signals, and find that CDK11 is required for self-renewal and pluripotency of hESCs. Cdk11 is known as a critical gene required for early mouse embryogenesis. However, its function in hESCs remains unexplored. We find that knockdown of CDK11 induces hESC differentiation and reduces the proliferation rate with little influence on cell survival, cell cycle and expression levels of core transcription factors. Interestingly, CDK11 knockdown activates FGFR signaling abnormally and the differentiation induced by CDK11 knockdown can be partially rescued by FGFR inhibitors, indicating that aberrant activation of FGFR signaling may, at least partially, account for hESC differentiation caused by CDK11 knockdown. Our result points out that abnormal activation of FGFR signaling could disrupt hESC self-renewal, although the signaling pathway is indispensable for hESC self-renewal. Taken together, this study uncovers an essential role of CDK11 in the maintenance of hESC self-renewal and proposes that the balanced level of FGFR signaling is critical to safeguard the hESC identity. The molecular mechanism by which CDK11 supervises the activation of FGFR signaling is currently under intensive investigation.

W-2013

GENERATION AND CHARACTERISATION OF HUMAN NAÏVE PLURIPOTENT STEM CELLS

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Comparative studies of pluripotency indicate that conventional human pluripotent stem cells (PSC) may represent post-implantation epiblast cells that are primed for lineage commitment. Our attention has focussed on capture of a more naive transcriptional and epigenetic state analagous to the mouse embryonic stem cell. We have established a simple transgene-free method for resetting human ESC or iPSC to naïve status via transient exposure to histone deacetylase inhibition. This chemical resetting protocol is effective across multiple embryo-derived and induced pluripotent stem cells and proceeds without karyotype changes. Furthermore, we show that human somatic cells can be reprogrammed directly to the naïve stem cell state and that naïve stem cells can be stably expanded in feeder-free culture. The global transcriptome profile of chemically reset cells clusters with that of embryo-derived naïve stem cells and diverges markedly from conventional human PSC. In addition to specific pre-implantation pluripotency factors, naive cells and human inner cell mass (ICM) show unique expression of transposable elements and their KRAB-ZNF regulators. Moreover, resetting to the naïve status is accompanied by global reduction in DNA methylation to the level observed in the ICM. Targeted hypomethylation potentially erases aberrant epigenetic marks that may compromise hPSC differentiation. In this context, we will report on the multi-lineage differentiation efficiency of reset cell lines compared to their primed pairs.

W-2015

SPATIAL SEGREGATION OF SOX2 AND NANOG DRIVES LOCAL EXTINCTION OF PLURIPOTENCY IN POSTIMPLANTATION MOUSE EMBRYOS

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Preimplantation pluripotency depends on the activity of a pluripotency gene regulatory network in which Sox2 and Nanog are key components. Although Sox2 and Nanog are expressed post-implantation, their roles in the post-implantation epiblast are unclear. Here we show that Sox2 and Nanog dynamically change expression during development from pre- to post-implantation stages, with declining levels overall, resolving into segregated protein expression domains. Strikingly, the Sox2-low, Nanog-high domain of the proximal posterior epiblast is the first region to lose pluripotency, being unable to produce teratocarcinomas or to establish pluripotent outgrowths in vitro. Pluripotent outgrowth formation can be rescued by constitutive expression of Sox2 but not Oct4. In postimplantation pluripotent cells, interactions between Nanog and Sox2 provide a mechanistic explanation of how segregated Nanog and Sox2 expression fields are set up to enable subsequent development.

W-2017

CONTINUOUS SUPPRESSION OF MEK1/2 IMPAIRS THE DEVELOPMENTAL POTENTIAL OF MOUSE EMBRYONIC STEM CELLS

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Simultaneous inhibition of Gsk3α/β and Mek1/2 activity in the presence of LIF (2i/L) induces a naïve state in mouse embryonic stem cells (ESCs) that resembles the inner cell mass (ICM) of the pre-implantation embryo. Since the ICM exists only transiently in vivo, it remains unclear how continuous propagation of naïve ESCs in vitro affects their stability and functionality. Here we show that extended culture of male ESCs in 2i/L results in the progressive erosion of genomic imprints, loss of H2A.X binding, and accumulation of chromosomal aberrations. Consistent with these observations, we find that the developmental potential of ESCs cultured in 2i/L is impaired. Mechanistically, we demonstrate that Mek1/2 inhibition is predominantly responsible for these effects, in part through downregulation of DNA methyltransferases. Additionally, we demonstrate that female ESCs cultured in conventional serum/LIF media phenocopy male ESCs cultured in 2i/L, including the aforementioned epigenetic and developmental abnormalities. Finally, we show that replacement of the Mek1/2 inhibitor with a Src inhibitor preserves the epigenetic and genomic integrity as well as developmental potential of ESCs. Taken together, our data suggest that, while suppression of Mek1/2 in ESCs maintains an ICM-like epigenetic state, continuous suppression results in irreversible changes that compromise their developmental potential.

W-2019

GMP-COMPATIBLE IPSC DERIVATION FROM MULTIPLE PERINATAL TISSUE SOURCES FROM THE SAME DONORS

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Side by side comparisons of starting material for generating iPSCs have been conducted but often focus on adult tissues or tissue types from donors of varying ages. We previously demonstrated that iPSCs can be generated from umbilical cord blood processed with manual or automated platforms at a cord blood bank. Next we sought to compare the reprogramming capacity of multiple newborn tissue types from the same donor. iPSCs were generated from mesenchymal stem cells isolated from thawed umbilical cord tissue, previously cryopreserved as a composite material, using an integration-free reprogramming method. The resulting cord tissue derived iPSCs were characterized and the reprogramming efficiency and line quality compared to the cord blood derived iPSCs generated from the same donor. iPSCs were successfully generated from matched cord blood and tissue pairs from each of 5 donors. iPSC derivation from cryopreserved cord blood required less starting material than cord tissue. However, the resulting cord blood and cord tissue derived iPSC lines were equivalent in quality based on a panel of tests including pluripotency gene expression profiling, embryoid body formation, plasmid loss and karyotype analysis. This study confirms that cord tissue cryopreserved as a composite material in a cord blood bank is a suitable alternative source material to generate iPSCs. Having identified that donor specific cord blood and cord tissue iPSCs are comparable in quality is of interest in that it provides rationale for iPSC generation from a newborn tissue source processed and stored under relevant cord blood banking regulations while preserving the cord blood unit in its entirety for future clinical utility.

W-2021

CHARACTERIZING CELL CYCLE SPEED FROM SINGLE CELL RNA-SEQUENCING OF STEM CELLS

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ESCs have a unique cell-cycle structure with rapid generation time that facilitates hyper-transcriptional state. At single cell level, expressional heterogeneity and noise within ESCs is intricately linked with cell cycle speed that ultimately define individual cell state. Consequently ESC differentiation is coupled with global cell cycle restructuring, changes in cell cycle speed and transcriptional programme. We performed single-cell RNA Sequencing (scRNA-seq) on FUCCI ESCs (Fluorescent Ubiquitination-based Cell Cycle Indicator) across two culture conditions (2i+LIF and serum+LIF) with precise cell cycle stage information (by index sorting) and computationally determined cell cycle speeds based on single cell transcriptomes.. We find that single cells across Serum+LIF condition have faster cell cycle speeds, increased heterogeneity and noisy expression compared to slow cycling, homogenous 2i-LIF cells. We identify subpopulations in serum-LIF conditions with increased differentiation propensity and higher cycling speed. To assess direct impact of cell cycle speed on transcriptional programme, we treated FUCCI ESCs with varying concentrations of CDK4/CDK6 inhibitors and performed index-sorted scRNA-seq on 1000 single cells. Alongside accumulation of cells in G1, we observed minor changes in total generation time without decreased viability. The significant reduction of cell cycle speed across both 2i+LIF and serum+LIF conditions was consistent with reduced transcriptional heterogeneity and correlated cell cycle stage determination. Psuedotime reconstruction captures single cell trajectories with distinct temporal transcriptome changes associated with cell cycle speed. We identify putative, novel cell cycle and chromatin regulators that respond earliest to inhibitors and hallmark discrete points in pseudotime trajectory. Global analysis of all cells uncovers distinct pattern of cell cycle speed linked to underlying transcriptional programme with varying differentiation propensity (Epiblast and primitive endoderm). Our work highlights how altering cell cycle speed provides an avenue to understand heterogeneity, underlying transcriptional state and ultimately cell state and its response to external cues.

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W-2023

DIRECT REPROGRAMMING OF SOMATIC CELLS INTO HUMAN INDUCED NAIVE PLURIPOTENT STEM CELLS, A NOVEL MODEL OF PREIMPLANTATION EPIBLAST

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Induced pluripotent stem cells (iPSC) have considerably impacted human developmental biology and regenerative medicine, notably because they circumvent the use of cells from embryonic origin and offer the potential to generate patient-specific pluripotent stem cells. However, conventional reprogramming protocols produce developmentally advanced, or primed, hiPSCs, restricting their use to post-implantation human development modelling. Hence, there is a need for hiPSCs resembling preimplantation naive epiblast. Here, we have generated naive hiPSCs (hiNPSC) directly from somatic cells of multiple donors using culture conditions supporting naive pluripotency in combination with the classical OKMS cocktail. We benchmarked these hiNPSCs against human preimplantation epiblast and reveal a remarkable concordance in their transcriptomic profiles, dependency on mitochondrial respiration and X chromosome status. hiNPSCs offer the potential to generate patient-specific counterparts of preimplantation epiblast, which will accelerate the understanding of pluripotency regulation throughout preimplantation development and will generate new opportunities for disease modeling and regenerative medicine.

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W-2025

HUMAN NAÏVE PLURIPOTENT STEM CELLS WITH GLOBALLY HYPOMETHYLATED GENOMES AND EPIGENETICALLY STABLE IMPRINTS POSSESS AUGMENTED NON-BIASED MULTILINEAGE DIFFERENTIATION POTENCY

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Several transgenic and chemical methods were recently reported that revert conventional human pluripotent stem cells (hPSC) to naïve-like states sharing transcriptional and epigenomic commonalities with mouse embryonic stem cells (mESC) and human pre-implantation embryos. However, some methods also led to chromosomal abnormalities and aberrant CpG hypomethylation at imprinted gene promoters with unclear downstream sequelae upon their functional pluripotency. We recently reported that chemical inhibition of GSK3, ERK and tankyrase signaling (LIF-3i) was sufficient for stable naïve reversion of a broad repertoire of >20 conventional hPSC lines. LIF-3i naïve hPSC (N-hPSC) maintained normal karyotypes, 2-4x fold decreases in global 5-methylcytosine CpG methylation activities, genome-wide CpG demethylation at ESC-specific gene promoters, dominant distal OCT4 enhancer usage, phosphorylated STAT3 signaling, and decreased ERK phosphorylation. LIF-3i induced expressions of naïve-specific human preimplantation epiblast genes (e.g., NANOG, KLF2, NR5A2, DNMT3L, HERVH). Methylation array analysis of >1400 known imprinted CpG sites in 12 independent LIF-3i N-hPSC revealed stability of imprints already established in isogenic conventional hPSC. In contrast to some reports of alternative naïve reversion methods resulting in loss of imprinted genomic regions, LIF-3i N-hPSC were devoid of systematic loss of imprinted CpG patterns or loss of DNA methyltransferase expression (e.g., DNMT1,3A,3B). LIF-3i naïve reversion resulted in decreased lineage-primed gene expression, with concomitant improvement in directed differentiation to all three germ layers. For example, LIF-3i N-hPSC generated PAX6+SOX1+Nestin+ neural progenitors more rapidly and efficiently than conventional isogenic hPSC counterparts. Improved differentiation efficiencies were similarly achieved for endodermal (CXCR4+FOXA2+) and mesodermal progenitors, including improved generation of vascular (KDR+CD73+ and ACE+) progenitors. LIF-3i N-hPSC will greatly impact studies in human pluripotency and regenerative medicine.

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W-2027

NON-CANONICAL BCOR-PRC1 COMPLEX REGULATES SELF-RENEWAL OF HUMAN ESCS VIA A NOVEL MECHANISM

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Polycomb group proteins are critical regulators of self-renewal and differentiation in many stem cell systems. They assemble into two complexes, PRC1 and PRC2, which act sequentially to establish repressive chromatin at the promoters of target genes. Core PRC1/2 subunits and their associated chromatin marks are found at the promoters of differentiation genes in ESCs. Surprisingly, depletion of PRC1/2 components in mESCs results in only a marginal increase in the expression of target genes with little or no effect on the self-renewal which indicates that PRC1/2 are dispensable for "naïve" pluripotency. In hESCs, which represent the "primed" pluripotent state the requirements for PRC1/2 have not been defined. We performed shRNA screen in hESCs to determine the requirements for PRC1/2 in self-renewal. We found that while PRC2 activity is dispensable for hESC maintenance, PRC1 function is critical for self-renewal. Indeed, simultaneous depletion of RING1A and RING1B results in a rapid loss of self-renewal and differentiation into endoderm and mesoderm. A similar phenotype was obtained when the PRC1 associated protein BCOR was depleted leading us to hypothesize that BCOR maintains hESC self-renewal via its association with the PRC1 complex. Biochemical and bioinformatics analyses revealed that BCOR co-precipitates with PRC1.1 complex proteins RING1A/B, PCGF1, KDM2B and SKP1 and co-localizes with PRC1/2 associated chromatin marks UbH2A and H3K27me3 at key differentiation genes. CRISPR/Cas9 deletion of BCOR led to a rapid dissociation of PRC1 complex from its genomic loci and activation of target genes indicating that BCOR plays a critical role in maintaining the integrity and the repressive activity of the PRC1.1 complex. Analysis of BCOR domain deletion mutants further revealed that while C-terminal region of BCOR is sufficient for the assembly of the PRC1 complex and its binding to targets, it is insufficient for the repression of BCOR target genes. Further studies identified N-terminal region of BCOR which exhibit potent repressive activity via the recruitment of accessory proteins that maintain highly compacted chromatin at the target sites. Our work identifies a novel mechanism of target recognition and repression by the BCOR-PRC1 complex which could be broadly applicable across the stem cell field.

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PLURIPOTENT STEM CELL DIFFERENTIATION

W-2031

ADVANCEMENT OF A CGMP-COMPLIANT IPSC-TO-MEGAKARYOCYTE DIFFERENTIATION PROTOCOL FOR PRODUCTION OF HUMAN PLATELETS

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Induced pluripotent stem cells (iPSCs) hold great promise for cellular therapy because of their potential to serve as an inexhaustible source for differentiation into any cell type in the human body. Platelets are among the most attractive cellular products of iPSCs because they are anucleate and can be irradiated prior to transfusion to eliminate any risk of tumorigenesis from iPSC-derived cells. Methods for safe and effective administration of platelets are already well established in the clinic, however, clinical platelet supply is severely limited by donor shortages, inconsistent quality, and a very short usable shelf-life of under 2 days. Previous proof-of-concept studies performed using research grade iPSC lines have demonstrated that human iPSCs can be effectively directed to differentiate into megakaryocytes (MKs), large cells that are each capable of producing hundreds of platelets. To produce donor-independent platelets applicable for clinical use, we are advancing a serum-free and feeder-free cGMP-compliant iPSC-to-megakaryocyte (MK) differentiation protocol. To this end, we first procured and tested several established cGMP-grade iPSC lines to identify an optimal source capable of generating large quantities of CD41+ megakaryocytic cells upon differentiation. The iPSC-derived MKs were assessed by biomarker expression, ploidy, cytoskeletal composition, morphology, ultrastructure, and platelet yield, and were found to compare favorably to primary MKs derived from human CD34+ umbilical cord blood or mobilized peripheral blood cells (used as a positive control). Functional testing of platelets derived from these iPSC-MKs is ongoing. We are currently optimizing our protocol to maximize MK yield and minimize cost of production, in a manner that is amenable for cGMP

manufacture at industrial scale for clinical purposes. Collectively, these studies demonstrate our continued advancement towards the cost-effective production of donor-independent platelets that will ultimately revolutionize transfusion medicine.

W-2033

DYNAMIC PROTEOME PROFILING OF DIFFERENTIATION AND REPROGRAMMING

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The profound transformations taking place during cellular reprogramming and differentiation occur on multiple levels and demand multi-scale, multi-disciplinary research efforts. To study these processes at a proteome-wide scale, we used a combination of ultra-high resolution mass spectrometry and downstream bioinformatic analysis to characterize global changes in protein expression during differentiation and subsequent reprogramming, within the same cellular system. To this end, mESCs and iPSCs derived from a mouse strain containing an inducible OKSM cassette and an Oct4-GFP reporter were differentiated to mature glutamatergic neurons, passing through a radial glia cell stage, and cells were collected at 6 different time points over 12 days for proteome analysis. At the final differentiation stage, the cells were 100% Oct4-negative, expressed neuron-specific markers and displayed mature neuronal morphology. This cell population was then subjected to pluripotency induction by activation of the OKSM-cassette until secondary iPSCs colonies were generated. Cells were FACS-sorted for Oct4-GFP expression at distinct time points, to distinguish populations that were primed for differentiation (switching off Oct4) and for reprogramming (switching on Oct4). To our knowledge, the proteomic dataset we generated from all collected and sorted cell populations presents a first-of-a-kind, high-throughput Omics study on a full cycle of cell-fate conversions. The dynamic expression profiles of thousands (>5600) of proteins during these transitions opens up a unique perspective into the temporal regulation of entire pathways and sheds light on the reciprocity of their activation and silencing during the opposing processes of differentiation and reprogramming. In addition, our findings lay a strong basis for the search of novel regulators of pluripotency gain and neural lineage specification. For example, we identified a cluster of ~90 proteins which have the same expression increase during differentiation and share highly enriched ontology terms for differentiation and neurological development. Interestingly, their expression was predicted to be regulated by only 7 transcription factor families, 13 members of which have previously not been associated with stem cell differentiation.

W-2035

CARDIOVASCULAR PROGENITOR CELL EXPANSION FOR DIFFERENTIATION INTO CARDIOMYOCYTES FOR REGENERATIVE CARDIAC THERAPY

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Endogenous and human induced pluripotent stem cell (hiPSC)-derived Cardiovascular Progenitor Cells (CPCs) have the ability for self-renewal, clonogenicity, and differentiation into endothelial cells, smooth muscle and most important for this project cardiomyocytes (CMs). After a myocardial infarction (MI) cells are damaged and adult CMs do not have the innate ability to repair the injury caused by a MI. The native myocardium has little to no regenerative capabilities so any loss of functional myocardial tissue is replaced by non-beating scar tissue which leads to weakening of the heart's pumping capacity, heart failure and eventually death. Current therapies lack the ability to restore cardiac tissue post-MI. Studies for regenerative therapy using autologous CPCs derived from the patient is currently a challenge. The proposed research uses well-characterized hiPSC-derived CPCs to assess ability to repair damaged myocardium post-MI in a large animal model. We have developed reproducible protocols to differentiate hiPSCs into CPCs and to expand these cells, eventually differentiating them into cardiomyocytes (CM), smooth muscle and endothelial cells in vitro. These CPCs have been characterized by the following surface markers Flk1, PDGFR, and TkrB. CPCs identified by these markers have shown potential to differentiate into CMs in vitro. The initial differentiation is an event that takes place throughout a 5-day span, Days 0-4. Throughout the differentiation process a base medium is used consisting of different growth factors. Once the iPSCs are at their desired confluency of 80%, Day 0 begins, from Day 0 to Day 4 the cells are exposed to a variety of growth factors that include the base medium in a series of induction stages and incubation. Day 3 culminates the induction and differentiation of CPCs, which is then followed by Day 4. Following differentiation, we have demonstrated that these CPCs have the ability to engraft into the myocardium of rats in a post-MI model and to differentiate into CMs in vivo, improving ventricular function. We plan next to expand this model into a porcine MI model.

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W-2037

ZINC REGULATES DIFFERENTIATION OF HESCS TO DEFINITIVE ENDODERM

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Differentiation of human pluripotent stem cells toward definitive endoderm (DE) is the critical first step for generating cells comprising organs such as the gut, liver, pancreas and lung. Many in-vitro DE differentiation protocols have been published with significant variations in conditions such as plating condition, differentiation initiation conditions, induction factors etc. But the final product from all protocols is always heterogeneous with a proportion of cells failing to differentiate properly and maintaining expression of pluripotency factors such as Oct4 and Nanog. To dissect the underlying mechanisms leading to the resistance of these residual "pluripotent" cells to DE differentiation, we employed single cell RNA-sequencing technology on cells collected at four time points during a 4-day DE differentiation. We identified high levels of metallothionein (MT) genes in the residual Oct4-positive cells. Using X-ray fluorescence microscopy and multi-isotope mass spectrometry imaging, we discovered that high MTs correlate with high levels of nuclear zinc in the Oct4-positive cells. By adjusting zinc concentration in the differentiation media, we were able to modulate DE differentiation efficiency — higher zinc levels resulted in less efficient differentiation and, conversely, lower zinc improved differentiation efficiency. This study improves our understanding of in-vitro DE differentiation and provides actionable options to improve DE differentiation efficiency.

W-2039

TARGETED EPIGENETIC EDITING REVEALS MECHANISMS GOVERNING THE SPECIFICATION OF INDUCED NEURONS

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The induction of specific cell types from pluripotent stem cells or differentiated cells via epigenetic reprogramming will advance applications in disease modeling, drug screening, and cell therapies. These cell types can be induced through the expression of master regulatory transcription factors that program the transcriptional networks that govern cell identity. Recently, we developed a strategy to reprogram fibroblasts to neuronal cells using epigenome editing tools repurposed from the CRISPR/Cas9 gene editing platform (Black et al., Cell Stem Cell 2016). This strategy entailed targeting and activating endogenous proneural genes within fibroblasts, rewriting the epigenetic marks at the target loci, and enabling stable autonomous expression of the target genes. Building on this work, our aim is to apply epigenetic editing tools to perturb transcriptional networks implicated in cell differentiation in order to develop improved methods to generate disease-relevant cell types. As a proof-of-principle demonstration, we utilized a CRISPR/Cas9-based transcriptional repressor, consisting of nuclease-null Cas9 fused to the KRAB heterochromatin-initiating domain (dCas9-KRAB). We employed dCas9-KRAB to silence endogenous transcription factors that were shown via RNA-sequencing to be activated upon induction of the proneural factor Ascl1 for reprogramming fibroblasts to neurons. We found that targeted repression of Zfp238 significantly attenuated reprogramming, as determined by a reduction in expression of the pan-neuronal markers β–Tubulin 3 and Map2. Zfp238 has been shown to play a role in neurogenesis during development, and future work will entail genomic analyses to further elucidate its role in the generation of induced neurons. In addition, to enable the high-throughput perturbation of transcriptional networks, we engineered an induced pluripotent stem cell (iPSC) line to express mCherry as a transcriptional reporter of β–Tubulin 3. This engineered cell line facilitates the isolation of differentiated cells from undifferentiated iPSCs via FACS. Ongoing work entails exploiting the scalable nature of the CRISPR/Cas9 system combined with our engineered iPSC line to perform pooled epigenetic screens to uncover transcriptional networks that modulate neuronal fate specification.

W-2041

EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO NEURAL PROGENITOR CELLS

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Neurological disorders are the leading cause of human morbidity, accounting for over ten percent of all human disease. Traditional approaches for studying nervous system development, injury, and disease are largely limited to animal models, which often fail to fully recapitulate human disorders. Moreover, the variable etiology of a given neurological disease in humans demands a personalized approach to understanding and treating individual patients. Human pluripotent stem cells, including embryonic and induced pluripotent stem cells, offer an essentially unlimited source of neural cells that can be used not only to study the mechanisms of human disease, but also as powerful tools for neural regeneration. Here, we introduce our StemXVivo® Neural Progenitor Cell Differentiation Kit,

which efficiently converts human pluripotent stem cells into neural progenitors. Within seven days, pluripotent stem cells are converted to Pax6-, SOX1-, and Nestin-positive neural progenitor cells. The derived neural progenitor cells can be expanded for several passages in vitro and differentiated into all three major cell types of the nervous system: neurons, astrocytes, and oligodendrocytes. These neural progenitors provide an intermediate multipotent stem cell population for further downstream differentiation, neuronal subtype derivation and mechanistic studies. The StemXVivo® Neural Progenitor Cell Differentiation Kit provides a powerful platform for the reproducible generation of neural progenitor cells from diverse pluripotent human stem cell sources for use in disease modeling and drug discovery.

W-2043

DERIVATION OF BLOOD-BRAIN BARRIER ENDOTHELIAL CELLS FROM PORCINE INDUCED PLURIPOTENT STEM CELLS

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Although porcine blood-brain barrier (BBB) models are more analogous to humans than rodents, only a few putative BBB models have been generated by co-culture of porcine primary cells. They are not readily available and lose some of their phenotypes when maintained in vitro for long-term culture. To establish improved in vitro porcine BBB models, we differentiated novel brain microvascular endothelial cells (BMECs) from porcine induced pluripotent stem cells (iPSCs) using a modified human-based protocol. Briefly, the dissociated single cells from iPSCs were seeded at 3 × 105 cells/cm2 in Geltrex. For differentiation, cells were maintained for 3 days of expansion and then switched to unconditioned medium (UM) lacking bFGF for 6 days. Then, we subcultured cells onto collagen/fibronectin coated plates and changed BMEC medium for 2-3 weeks. About two weeks later, we observed a cluster of round cells surrounded by spindle-shaped adherent cells termed as colony-forming units (CFU) of putative BMECs. Over time, the cluster of cells disappears and remained adherent spindle-shaped cells showed properties of endothelial cells. Although further study will be needed, porcine BMECs established in the present study would be a great source to study comparative anatomy and physiology of porcine versus human in vitro BBB.

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W-2045

A TRANSCRIPTOMIC APPROACH TO IDENTIFY POTENTIAL MARKER GENES FOR PREDICTION OF DIFFERENTIATION PROPENSITY OF HUMAN INDUCED PLURIPOTENT STEM CELL LINES

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Human induced pluripotent stem cells (hiPSCs) have the ability to differentiate into a variety of cells and to self-renew in vitro. Because of these two characteristics, hiPSCs have been expected to provide new applications for regenerative medicine/cell therapy. Although various in vitro differentiation protocols have been developed for efficient derivation of specific cell types, hiPSC lines vary in their ability to differentiate into specific lineages. Therefore,surrogate biomarkers that accurately predict the differentiation propensity of hiPSCs could be helpful for the development and manufacture of hiPSC-derived differentiated cells for therapies or in vitro assays. In the present study, we tried identifying the genes that potentially predict the differentiation propensity of hiPSCs into three germ layers. At the first step, we obtained comprehensive mRNA expression profiles of ten hiPSC lines, using microarray analysis. Next, we induced bias-free differentiation of all the hiPSC lines by embryoid body formation to acquire the expression profiles of germ layer marker genes. To quantify a differentiation propensity for three germ layers, the first principal component scores of the post-differentiation gene expression data were calculated by principal component analysis. Subsequently, Spearman's rank correlation coefficients between the gene expression at the undifferentiated state and the principal component scores of the embryoid bodies were determined, identifying 308 genes (probes) significantly correlated with the differentiation propensity into the germ layers Furthermore, loss/gain-of-function experiments showed that one of these genes were functionally involved in the germ layer differentiation. These results suggest that our approach is simple and useful to identify potential biomarkers for the selection of the suitable cell line for manufacture of a specific cell-based product, as well as to find novel factors to elucidate the molecular

mechanism of the differentiation of pluripotent stem cells.

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W-2047

YAP1 SAFEGUARDS MURINE EMBRYONIC STEM CELLS FROM EXCESSIVE APOPTOSIS DURING DIFFERENTIATION

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Apoptosis plays critical roles in the regulation of differentiation, development, and prevention of tumorigenesis. Numerous signaling pathways tip the balance between survival and programmed cell death. In particular, the Hippo pathway, which is mutated in a plethora of cancers, may promote or inhibit apoptosis in a context-dependent manner. We have found that deletion of Yap1, a Tead-mediated enhancer activating protein, significantly increases programmed cell death in murine embryonic stem (ES) cells during differentiation. This is rescued by treatment with Z-VAD-FMK, a pan-caspase inhibitor. However, neither necrostatin, a Ripk1 inhibitor, nor modulation of autophagy attenuate cell death, implying that loss of Yap1 sensitizes ES cells to caspase-dependent apoptosis rather than activating an alternative cell death pathway. Indeed, Yap1 -/- cells undergo greater cleavage of caspases 8 and 3 as well as Parp1 and Bid compared to wild type (WT) cells, and they are more sensitive to cytotoxic stimuli. Transcriptional profiling reveals that Bcl-2 is dramatically upregulated in WT cells, but this upregulation is far weaker in Yap1 -/- cells. Preliminary BH3 profiling shows that ES cells in the early stages of differentiation, but not undifferentiated cells, are susceptible to pharmacological inhibition of Bcl-2 and related proteins in a dose-dependent manner. Several ChIP-Seq data sets published by other groups indicate that Yap1 occupies a putative regulatory element of Bcl-2 in cardiomyocytes and satellite cells, and this same site is occupied by p300, which can predict enhancer regions, in embryonic ectoderm. Collectively, our results suggest that Yap1 safeguards ES cells during differentiation by attenuating apoptosis through activation of anti-apoptotic genes, particularly Bcl-2. We propose to perform our own ChIP-qPCR in differentiated ES cells using Yap1 antibody for immunoprecipitation and further characterize other pro- and anti-apoptotic genes implicated in the mechanism using genetic and pharmacological methods.

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W-2049

DECELLULARIZED LIVER EXTRACELLULAR MATRIX (DLM) MEDIATED HEPATIC DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSC)

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Liver tissue engineering has emerged as a promising approach in organ transplantation, but has been hampered by the lack of a reliable and readily available cell source. Several characteristics highlight human induced pluripotent cells (hiPSC) as a desirable source, including their differentiation potential, ability to self-renew and the possibility of making patient specific cells. We developed a tissue decellularization protocol that efficiently removes cellular material as demonstrated by low DNA content, while retaining a high degree of extracellular matrix components, including collagen and glycosaminoglycans. Subsequently, hIPSC were differentiated on the decellularized liver matrix (DLM) scaffolds using an established hepatic differentiation protocol. We demonstrated that using DLM leads to significant upregulation of functional hepatic markers (Albumin, CYP3A4, CYP2B6, CYP2C8, and CYP2D6) when compared to standard differentiation conditions (hESC-qualified matrigel). In addition, expression of a number of hepatic transcription and nuclear factors were found to be within levels comparable to those of primary human hepatocytes. Analysis of progression of differentiation on DLM demonstrated that hepatic developmental marker expression was more consistent with in vivo hepatic development comparted to matrigel. The DLM-derived cells exhibited key hepatic characteristics - morphology, bile canaliculi, glycogen storage, albumin expression and secretion and cytochrome P (CYP3A4) activity - that were comparable to those observed in primary neonatal human hepatocytes. We investigated the appropriate timing for the introduction of DLM into the differentiation protocol, and found that best results are obtained when cells are plated on DLM since the earliest stage of differentiation, and accompanied by a progressive loss of sensitivity to substrate composition at later stages. The results presented in this study suggest that that liver ECM provides specific cues that aid with hepatic differentiation of hIPSC. The significance of this work is that it allows for the development of differentiation protocols that take into account signals from ECM, hence, closely recapitulating of the in-vivo microenvironment and resulting in cells that are phenotypically closer to mature hepatocytes.

W-2051

STEMDIFF™ HEMATOPOIETIC KIT REPRODUCIBLY GENERATES FUNCTIONAL HEMATOPOIETIC PROGENITOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Hematopoietic cells generated from human pluripotent stem cells (hPSCs) can be used to model blood diseases and as an alternate source of blood cells for transplantation. However, robust methods to differentiate hPSCs to hematopoietic progenitor cells (HPCs) have been difficult to develop. The STEMdiff™ Hematopoietic Kit reproducibly generates HPCs from multiple human embryonic stem cell (H1, H9) and induced pluripotent stem cell (WLS-1C, STiPS-F016, STiPS-M001, STiPS-B004) lines under serum- and feeder-free conditions. To do this, hPSC aggregates were plated on Corning® Matrigel® in TeSR™ medium. The cells were then sequentially incubated in two differentiation media from the kit, and harvested on day 12 for analysis. Results show that CD34+CD45+ HPCs were detected at 41 ± 2% (mean ± SEM, n=69) in the day 12 supernatant, with an average yield of 466,500 ± 41,000 HPCs per well of a 12-well plate. The frequency of hematopoietic colony-forming units (CFUs), as assayed in Methocult™ medium (H4435), was 119 ± 10 CFU/10^4 supernatant cells (n=63). To assess the developmental stage of the HPCs, globin transcripts of pooled erythroid colonies were measured by qPCR. Both embryonic and fetal globin were highly expressed (n=3), consistent with embryonic or early definitive stage hematopoiesis. Further characterization indicated that >94% supernatant cells expressed CD43 (n=8), a pan-hematopoietic marker expressed in early human development. When assessed for the hematopoietic stem and progenitor cell phenotype CD34+CD38-CD45RA-CD90+CD49f+CD45+, expression was variable with 0.8 to 17% of cells expressing this phenotype (n=3). The key transcription factors SCL, RUNX1, GATA2, GATA1, and LMO2 were measured by qPCR and were all highly upregulated compared to hPSCs (n=8). As a proof-of-principle, these hPSC-derived HPCs were further expanded and differentiated to the erythroid lineage using StemSpan™ SFEM II medium with Erythroid Expansion Supplement. The 4 hPSC lines tested showed 8- to 56-fold expansion of total cell number over 14 days. At the end of the 14-day culture, all hPSC lines had high purity of erythroid progenitors with 79 - 95% CD71+GlyA+ cells. In summary, the STEMdiff™ Hematopoietic Kit reproducibly differentiates hPSCs to functional HPCs expressing key cell surface markers and transcription factors.

W-2053

DEVELOPMENT OF REAL-TIME IMAGING ASSAYS FOR TOXICITY PREDICTION USING HUMAN PLURIPOTENT STEM CELL-DERIVED NEURAL STEM CELLS IN CHEMICALLY DEFINED CULTURE CONDITIONS

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Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), could be a promising new tool for in vitro developmental neurotoxicity (DNT) testing. Several DNT testing methods based on hESCs have been proposed with "3R" concept; refining, reducing and replacing the DNT tests on vertebrata. However, these methods contain steps of multi-cellular aggregation (embryonic bodies or neurospheres) and/or culturing with ill-defined or variable animal-derived materials such as fetal bovine serum (FBS), Knockout serum replacement (KSR), B27 supplement, or Matrigel. Such conditions hamper accurate monitoring cellular response to drug exposure and reduce reproducibility. Further, usage of animal-derived materials goes against 3R concept. More cost-effective, rapid and simple alternative method aiming for 3R concept should be expected. To address these issues, we developed a new method for directed induction of hPSCs to neural stem cells (NSCs) and imaging assays using the hPSC-derived NSCs using chemically defined medium consisting of minimum components, in adherent monolayer culture. The NSCs derived by our new method were validated by their cell morphology, protein expression profile, and potency of further neural maturation. In this study, we performed imaging assays using valproic acid (VPA) to verify whether the hPSC-derived NSCs could be suitable for usage on DNT testing. As a result, the influence of VPA was detected at low dose. The culture condition consisting of minimum essential components enables us high sensitive detection of the drug toxicity/efficacy. Further, it might be possible to predict toxic potencies in humans by calculating toxicity reduction by components in blood serum such as albumin from results on our cell-based assay. Thus, our methods can be applied to a high-sensitive, rapid and simple drug testing to predict drug toxicity effect on early neural differentiation as a part of DNT.

W-2055

CHARACTERIZATION OF HUMAN EMBRYONIC STEM CELL DERIVED GABAERGIC INTERNEURON PROGENITORS IN VITRO AND IN THE EPILEPTIC BRAIN

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The selective loss of GABAergic inhibitory interneurons creates an electrical imbalance in the hippocampal and cortical neural circuit and characterizes numerous neurodegenerative diseases. Our long-term goal is to replenish these inhibitory interneuron subtypes using an embryonic stem cell (ESC) source. During embryonic development, these inhibitory interneuron progenitors arise predominantly from a transient ventral forebrain structure known as the medial ganglionic eminence (MGE) and are characterized by the expression of NKX2.1. Cells for transplantation are generated from human ESCs using an adherent monolayer protocol and sonic hedgehog treatment. Human ESC-derived interneuron progenitors transplanted into the mouse hippocampus of NSG epileptic mice mature to express the neuronal markers Hu, NeuN, and the inhibitory neurotransmitter GABA by twelve weeks post-transplantation. In addition, mice with transplanted cells exhibited significant improvement in the Morris Water Maze spatial memory task by six weeks post-transplant. Patch clamp analysis indicates that hESC derived neurons are capable of firing mature action potentials following long-term in vitro culture and post transplantation into a mouse host. hESC-derived interneuron progenitors expressing channel rhodopsin eYFP are able to promote a blue light-mediated increase in inhibitory post synaptic current (iPSCs) in vitro. Preliminary analysis of the potential of transplanted cells to suppress recurring seizures in a mouse model of temporal lobe epilepsy suggests no significant seizure suppression. We are currently examining the potential of transplant-derived neurons to suppress the activity of excitatory granule cells in vivo and attempting to identify neuronal targets of the transplanted neurons.

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W-2057

MODELING OF HUMAN SOMITE PATTERNING USING IPSC

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Somite patterning is a significant event for vertebrate development. In response to signals such as WNTs, BMPs and SHH secreted from surrounding tissues, somite give rise to myotome, sclerotome, dermatome and syndetome, which in turn, are the origins of skeletal muscle, axial skeleton, dorsal dermis and axial tendon/ligament respectively. Therefore, induction of somite from hiPSCs is a critical step to harness pluripotent stem cells(PSCs) toward applications such as regenerative medicine and disease research of orthopedic surgery field. However, the efficient and reliable induction of somite remain a major challenge, also nobody has ever succeeded to generate dermatome and syndetome. In our study, we tried to recapitulate human somite patterning in vitro by mimicking the signal conditions during chicken/mouse somite development, and finally succeeded to generate the complete set of somite derivatives through presomite(PSM) state and then somite(SM) state in a stepwise manner. It indicates that the knowledge of mouse/chicken somite development was successfully applied to induction of human somite by using hiPSCs. Our novel induction method serves as an important foundation for the directed differentiation of dermis and tendon/ligament cells from PSCs. Also we're planning to apply this induction method toward FOP(Fibrodysplasia Ossificans Progressiva) study, in addition, toward tendon/ligament injury model.

W-2059

IDENTIFICATION OF CELL SURFACE MARKERS ON HUMAN LUNG PROGENITOR CELLS EXPRESSING NKX2-1

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The mortality rate of infants with pediatric lung diseases is 16.2% in the US. One condition, neonatal respiratory distress (RDS) syndrome, is caused by the insufficient production of pulmonary surfactant in distal lung epithelial cells, the alveolar type II cells (ATII), which leads to increased surface tension at end expiration, and occurs in preterm infants with arrested lung development. Cystic fibrosis is a proximal lung epithelial in disease that currently has no cure and affects 30,000

individuals. These diseases are difficult to study because the lung has 40 different cell types which are difficult to isolate and obtain from a human patient. Human induced pluripotent stem cells (hiPSCs) enables the study of particular cell types, and although the lung is a heterogenous tissue, specific cell types can be isolated by surface marker expression and then studied in vitro. Our hypothesis is to differentiate lung progenitor cells (LPCs) from hiPSCs in order to study early lung development, and discover surface markers that define critical lung cell populations that reside in the proximal and distal portions of the lung. Using published and novel protocols for embryonic lung development, we differentiated hiPSCs into definitive endoderm, anterior foregut endoderm and lung progenitor cells (LPC) using a cocktail of growth factors and small molecules. LPCs express high levels of NKX2-1 and this expression correlates with increased expression of the cell surface antigen Carboxypeptidase M (CPM). To isolate LPCs for further lung differentiation, we used positive selection for NKX2-1/CPM expressing cells through the use of a multifaceted comparative proteomic approach. We used in vitro screening of 300+ cell surface antigens in collaboration with the UHN monoclonal antibody core facility to discover surface markers that co-expressed CPM. The "top hits" generated will be further explored in order to characterize distal and proximal lung tissues derived from LPCs. We will then use RNA seq to define the genetic signature of the sorted cell types using the novel surface markers which will allow the creation and isolation of specific lung cells in which to study various pediatric lung diseases.

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W-2061

DYNAMIC CHANGES IN THE APOPTOTIC MACHINERY ON DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TO NEURONS

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Recent studies have shown that the thresholds to undergo apoptosis can be strikingly different in different cell types. For example, while undifferentiated human embryonic stem cells (hESCs) are highly sensitive to DNA damage-induced apoptosis, postmitotic neurons are highly resistant. These differences in sensitivities to apoptosis are physiologically important, as it permits for the rapid elimination of mutant hESCs in the developing embryos while maintaining the long-term survival of neurons. However, the exact mechanisms by which the distinct apoptotic thresholds are set in different cell types remains unknown. We used the model of hESC differentiation to identify the dynamic changes in apoptotic machinery as the undifferentiated hESCs become differentiated into postmitotic neurons. A critical mediator of apoptosis is Bax, which is present in the cytosol of most of the cells in an inactivated state. Upon apoptotic stimuli, Bax is activated and translocates to the mitochondria to induce apoptosis. We reported that in contrast to other cell types, hESCs are primed to undergo apoptosis as Bax is maintained in a constitutively active state localized to the Golgi in undifferentiated hESC's; DNA damage triggers the Golgi-to-mitochondrial tranalocation of active Bax to induce rapid apoptosis. Interestingly, within two days of differentiation, Bax is no longer maintained in an active state and the cells are no longer capable of undergoing rapid cell death. This novel discovery urged us to examine the status of the other key apoptotic factors as the hESCs cells are differentiated into neurons. hESCs (H9) were differentiated into nociceptor neurons using the Studer protocol and successful differentiation was confirmed by immunofluorescence and quantitative real time PCR for the markers of pluripotency and differentiation. Importantly, we examined the status of the key apoptotic proteins at the various stages of hESC differentiation. Our study of the expression pattern of various apoptotic factors and the correlation to cell death sensitivity of cells with hESC differentiation provides insight into the dynamic mechanisms by which distinct apoptosis thresholds are established with hES cell differentiation into neurons.

W-2063

DIFFERENTIATION OF HIGHLY-ENRICHED MIDBRAIN SPECIFIC DOPAMINERGIC NEURONS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSCS) ON FEEDER-FREE CULTURE

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Although iPSC-derived dopaminergic neurons are expected one of the potential sources for cell replacement therapy in Parkinson's disease (PD), efficiency of dopaminergic neuron induction from human iPSCs by current protocols is relatively low and several technologies including FACS sorting are

necessary to induce highly-enriched dopaminergic neurons for regenerative medicine. Recently, we have developed a neurosphere-based culture system to control the regional identity of hiPSC-derived neural progenitors by using small molecules. This system facilitates effective induction of midbrain specific neural progenitors that give rise to dopaminergic neurons. In this study, we tried to fit this system into feeder-free cultured iPSCs to obtain midbrain specific dopaminergic neurons for regenerative medicine. To examine midbrain specificity of neurospheres derived from feeder-free iPSCs, we performed real-time PCR to detect midbrain specific makers. EN1, which is expressed in the midbrain and anterior hindbrain, and TH, which is the dopamine-synthesizing enzyme and these markers were highly expressed in neurospheres treated with 3μM CHIR99021, a GSK3 inhibitor. Then, these neurospheres with midbrain identity were differentiated into dopaminergic neurons in an attached culture condition. Approximately 60% of Tuj-1 positive neurons were TH-positive dopaminergic neurons, suggesting that highly enriched dopaminergic neurons could be induced from feeder-free iPSCs by optimized culture condition without cell sorting or fluorescent reporters. We are trying to transplant these midbrain specific neurospheres into the striatum of 6-OHDA-lesioned PD model mouse to examine whether these midbrain specific neural progenitors are effective for PD symptoms.

W-2065

VENTRICLE-SPECIFIC CARDIOMYOCYTE DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

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Acute myocardial infarction leads to loss of large number of cardiomyocytes causing severe ventricular tissue damage. Pluripotent stem cells (PSC) are gaining popularity as the ideal source to regenerate the lost cardiomyocytes, due to their capability to proliferate and differentiate into cardiac lineage. Various differentiation protocols have been established to differentiate PSC into cardiomyocytes, however all resulted in the development of a heterogeneous (atrial, nodal and ventricular) population of cardiomyocytes. Unfortunately, once transplanted to the left ventricle, these nodal cells will generate unwanted ventricular arrhythmias. We aimed to design a protocol to differentiate mouse embryonic stem cells (mESC) into ventricle-specific cardiomyocytes. By inhibiting Wnt signaling in the early stage, we aimed to trigger mESC to develop into cardiomyocyte lineage while inhibition of Retinoic acid signaling in the later stages would trigger a ventricular phenotype. To enhance the differentiation, ascorbic acid was added throughout the process. Gene expression of stage specific cardiomyocyte markers was validated by qPCR and protein expression was validated by flow cytometry and immunofluorescence analysis after 14 days of differentiation. Our preliminary results showed that all the major cardiac markers (Isl-1, Nkx2.5, Mef2C, CTnT, IRX4, and MLC2V) were expressed in a stage specific manner. Importantly, ~77% of cells were expressing the ventricle specific IRX4 after 14 days of differentiation (n=3). Our approach is the first step to the development of a differentiation protocol for ventricle-specific cardiomyocytes, which will have a significant contribution to the field of cardiac regeneration therapy.

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W-2067

WIP1 DIRECTLY DEPHOSPHORYLATES NLK AND INCREASES WNT ACTIVITY DURING GERM CELL DEVELOPMENT

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Defective testis development and reduced spermatogenesis are part of the distinct phenotype of Wip1 null mice. However, the molecular mechanism underlying this aberrant testis physiology remains unknown. Herein, we show that Wip1 expression was closely associated with Wnt activity in a β-catenin-independent manner. Wip1 directly interacted with Nemo-like kinase (NLK) and dephosphorylated its activating phosphorylation site, which retains close homology with p38 MAPK, a well-known Wip1 substrate. Inhibition of NLK activity by Wip1 markedly decreased the phosphorylation of Lymphoid Enhancer-Binding Factor 1 (LEF1), thereby disrupting its interaction with β-catenin. Notably, depletion of Wip1 significantly impaired the germ cell development, determined by re-expression of Oct4 and germ cell marker such as Ddx4, Nanos3 and Dnd1 during germ cell development from OG2 (Oct4-GFP transgenic) mouse embryonic stem cells. Re-expression of Wip1, which was dramatically decreased after differentiation from mESCs, was manifested in parallel with the expression of germ cell development markers and SRY-Box 17 (Sox17), a downstream target of Wnt. This suggests that Wip1 expression is important for germ cell development through Wnt activity.

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W-2069

SINGLE CELL-DERIVED HUMAN INDUCED PLURIPOTENT STEM CELL DIFFERENTIATION TO RED BLOOD CELLS

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Donor-derived red blood cells (RBCs) are the most common form of cellular therapy. However the source of cells dependent on donor availability with a potential risk of allo-immunization and blood borne diseases. An immortal source to produce in vitro cultured RBCs, such as iPSC would provide an autologous product with absence of immune reactions. The in vitro production of iPSC-RBC has proven to be possible, however there are barriers to overcome prior to clinical application. e.g. virus- and transgene-free iPSC generation, xeno-free culturing methods, scale up cultures to obtain transfusion units (1-2*10^12 erythrocytes). To solve the above mentioned issues a customized humanized GMP-grade medium (Cellquin) was generated in order to control erythroid culture parameters and to reduce culture costs. This medium allowed 1*10^8 times erythroid expansion from PBMCs to pure adult erythroblast (EBL) cultures within 25 days, comparable to non-GMP commercial media. Non-integrative polycistronic episomal vector containing (OCT4-SOX2-KLF4-cMYC-LIN28) was used to reprogram PBMC-expanded EBLs to iPSC, displaying pluripotency potential and normal karyotype. iPSCs were adapted to single cell dissociation allowing directed colony differentiation using a feeder-free monolayer approach. From day 6 of differentiation Cellquin was applied with lineage-specific growth factors, resulted iPSC differentiation to EBLs which was initiated by the appearance of hemogenic endothelium following hematopoietic specification. Our differentiation method resulted in ~1*10^5 fold expansion to CD41- CD34- CD71+ CD235+ CD36+ EBLs within 21 days (12 days iPSC diff + 9 days EBL expansion), which was reproducible using different iPSC lines. Further maturation of iPSC-EBLs yielded CD71+ CD235+ CD36- pure orthochromatic normoblasts expressing mainly gamma globin chains (fetal ~85%), small amount of beta- (adult <~5%) and epsilon- globins (primitive ~10%). Currently we are testing enucleation potential of matured iPSC-EBLs. In conclusion, we showed that our monolayer differentiation approach is simple, highly controlled, robust and compatible with up-scaling. Avoiding virus-, integrative reprogramming, feeders and with our GMP-grade media we maintained a cost effective system moving toward clinical application.

W-2071

INVESTIGATING THE MITOCHONDRIAL DISULFIDE RELAY SYSTEM IN HUMAN PLURIPOTENT STEM CELLS WITH MITOBLOCK6

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MitoBloCK6 (MB-6) was identified in a screen for inhibitors of the protein Augmenter of Liver Regeneration/Growth Factor Erv1-Like (ALR/GFER). ALR is a taxonomically conserved protein involved in the disulfide relay system, which facilitates import of cysteine rich proteins into the mitochondrial intermembrane space (IMS). Previous studies have shown that MB-6 causes apoptosis in human pluripotent stem cells (hPSCs) while leaving their differentiated derivatives intact. Our current work aims to determine the mechanism for MB-6 rapid cell death in hPSCs. Toward this goal, we have identified the time of differentiation, which differs based on lineage and tissue type, at which hPSC derivatives become resistant to MB-6 exposure. Antioxidants mitoTEMPO and N-acetylcysteine had little effect on hPSC death after MB-6 treatment, suggesting that reactive oxygen species (ROS) influence on the apoptotic mechanism is minimal. We hypothesize that MB-6 causes preferential death of hPSCs through differences in ALR protein import activity, and that these differences are mediated by differential protein interactions in pluripotent versus differentiated cell states. To examine this hypothesis, we are utilizing immunoprecipitation in combination with mass spectrometry (IP-MS) to identify changes in ALR interactions between undifferentiated hPSCs and their differentiated progeny. We anticipate new insights into differences in mitochondrial function in pluripotent versus differentiated cells. In addition, MB-6 could become a tool to remove pluripotent cells that fail to differentiate into the desired cell types, furthering clinical stem cell applications.

W-2073

AN IMPROVED METHOD TO DIFFERENTIATE HUMAN PLURIPOTENT STEM CELLS TO ADULT-LIKE SKELETAL MUSCLE

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Differentiated human pluripotent stem cells have become an invaluable tool to study development and disease states as they are usually more relevant to human pathologies than animal models and immortalized cells. In addition, they provide an unlimited, uniform resource to develop cell-based primary and secondary phenotypic assays for drug development. However, a common limitation is that the differentiated cells do not reach a truly adult-like state and remain more embryonic in their nature. Developing robust differentiation protocols addressing this issue is expected to further increase usefulness of hPSCs. Our interests at Genea Biocells include development of stem cell models for muscle disorders. Previously, we developed and published a fast, robust and highly efficient differentiation method for skeletal muscle that does not rely on the overexpression of myogenic transcription factors or cell sorting to enrich myogenic populations (Caron et al. 2016). Both our method, as well as reported alternatives, yield elongated myotubes with limited multinucleation typical for embryonic muscle vs. primary adult muscle cultures exhibiting a thick, branched morphology with many nuclei per cell. In an effort to optimize our differentiation protocol further, we conducted small molecule screens and identified series of pharmacologically defined, lead-like small molecule hits - compounds that 1) further improve the number of MyoD-positive myoblasts and 2) enhance myoblast fusion to large multinucleated myotubes resembling primary cultures. We also show that these compounds make several growth factors redundant in the new generation media which were previously included for efficient differentiation. We are now conducting a detailed functional and molecular characterization of these myotubes, and we are testing their suitability to model a panel of muscle diseases.

W-2075

IDENTIFICATION OF BMI(I) ON L-TYPE CA2+ CHANNELS IN HESC-DERIVED CARDIOMYOCYTES

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Human embryonic stem cells (hESCs) were differentiated to cardiomyocytes followed by metabolic selection to enrich differentiated cardiomyocytes and eliminate undifferentiated cells. 30 day differentiation and selection protocol generated highly-pure and well-differentiated cardiomyocytes from hESCs according to the analysis by immunocytochemistry, flow cytometry, and RT-PCR analysis. Highly-pure cardiomyocytes derived from hESCs showed the expression of mature cardiomyocyte markers, such as cTnT, MLC-2A, and α-SA. In addition to the expression of cardiac marker genes, the function of specific ion channels expressed in cardiomyocytes was evaluated to confirm the successful differentiation, which can contribute to establishing the protocol for assessing the pharmaceutical efficacy of hESC-derived cardiomyocyte in field of cardiac research. In this study, we identify the Na+, Ca2+ and K+ channels and recorded action potential in hESC-derived cardiomyocytes. In addition, we investigated the effect of bisindolylmaleimide (BIM) (I), which inhibits native cardiac Ca2+ channels, on hESC-derived cardiomyocytes. The difference of sensitivity between native cardiac cells and hESC-derived cardiomyocytes was present. The inhibitory effect of BIM (I) on L-type Ca2+ channels was more sensitive in hESC-derived cardiomyocytes than cardiomyocytes of rat ventricular cells, which suggests that hESC-derived cardiomyocytes is a superior platform for screening drugs for human application. Although the hESC-derived cardiomyocytes have several limitations for clinical application, hESC-derived cardiomyocytes can be utilized for the development of novel pharmaceuticals and screening of cardiotoxicity.

W-2077

THE CIRCADIAN CLOCK DEVELOPMENT DURING HEPATIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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Circadian rhythms are daily cycles of physiology and behaviors that are driven by an endogenous oscillator with a period of approximately (circa) one day (Diem). This system is fundamental to achieve temporal homeostasis with the environment at the molecular level, by organizing physiology and behavior in a proactive rather than a responsive manner. During development, the environmental conditions surrounding and affecting the embryo, such as the availability of nutrients or the concentration of metabolites, change throughout the day. The embryo does not yet possess a mature functional clock able to anticipate these variations; the development of such mechanism is accomplished during gestation. It is clear that in mammals circadian rhythmicity develops gradually during ontogenesis, but the mechanisms involved at cellular level during differentiation of embryonic stem cells are still unknown. The characterization of the circadian system and its complex network of interactions with relevant physiologic and pathologic pathways is in continuous evolution.Up to now, the knowledge of clock ontology and development and its relevance during cellular differentiation is restricted to a very few articles. This work represents the first attempt of investigating the cell-autonomous circadian clock onset in human pluripotent stem cell during hepatic differentiation. Human pluripotent stem cells differentiated in microfluidic environment achieved sustained expression of hepatic markers and of most of circadian genes (Rev-Erb alpha, Bmal1, Clock, Per2, Per1, Cry1 and Cry2). Only after the stage of definitive endoderm circadian rhythmicity of 24h cycles start to be observed in most of circadian genes. The circadian onset seems to be correlated with the daily variation of metabolic signals in which the microfluidic cell culture is exposed.

PLURIPOTENT STEM CELL: DISEASE MODELING

W-2079

INDUCED PLURIPOTENT STEM CELL CARDIOMYOCYTES FOR THE STUDY OF VARIANT PATHOGENICITY IN LONG QT SYNDROME (LQTS)

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Long QT syndrome (LQTS) is a condition with monogenic inheritance for which 30% of patients are without a genetically-confirmed diagnosis. Rapid identification of causal mutations in LQTS, a potentially fatal condition, is a priority to ensure optimal management of cardiac events and to reduce mortality. Sequencing technologies are useful for the identification of potentially novel causal genes and mutations; however, they come with significant challenges in data interpretation. LQTS could therefore benefit from having a platform for evaluating the functional impact of candidate variants in the physiological context of human cardiomyocytes. Using a variant in Kir2.1 (Gly52Val) revealed by whole exome sequencing in a patient presenting with symptoms of Andersen-Tawil syndrome as a proof of principle, we demonstrate that commercially available isogenic human induced pluripotent stem cell derived cardiomyocytes (hiPS-CMs) are a powerful model for the screening of variants involved in genetic cardiac diseases. Indeed, confirming results previously obtained using HEK cells, immunohistochemistry experiments and whole-cell current recordings in hiPS-CMs expressing the wild-type or the mutant Kir2.1 demonstrate that Kir2.1-52V alters channel cellular trafficking and fails to form the fully functional ion channel. Moreover, Kir2.1-52V is associated with lower excitability, dramatic prolongation of action potential duration with evidence of arrhythmic activity, parameters which could not have been studied using HEK cells. Our study indicates the molecular mechanism underlying the effect of Kir2.1-52V responsible for the clinical manifestations observed in the patient also than provides evidence for the use of isogenic hiPS-CMs as a physiologically relevant model for the screening of variants.

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W-2081

THE IMPACT OF CYTOPLASMIC ASPARTYL-TRNA SYNTHETASE MUTATIONS ON HUMAN IPSC DERIVED NEURONAL CELL TYPES

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The role of aminoacyl-tRNA-synthetases (ARS) is to accurately combine tRNAs with their corresponding amino acids and therefore facilitate correct protein synthesis. Interestingly, identified ARS-mutations lead to diseases of the nervous system with disruptions in myelination. One of these rare and poorly understood diseases is Hypomyelination with Brain stem and Spinal cord involvement leading to Leg spasticity (HBSL) induced by mutations in the cytoplasmic aspartyl-tRNA synthetase (DARS). Due to their central role in myelination, oligodendrocytes are the primary focus of research in HBSL. How different mutations of DARS specifically affect oligodendrocyte function and their myelinating ability is to date unknown. To achieve insights into HBSL pathology, footprint-free induced pluripotent stem cells from four HBSL patients were generated. They display increased spontaneous differentiation, a prolonged G1 and reduced S1 Phase, additionally are prone to heightened apoptosis in vitro. We hypothesise that the defective protein synthesis due to DARS mutations leads to ER stress via the unfolded protein response. Interestingly, aspartate supplementation partly rescues increased apoptosis as measured by TUNEL assay and reduces mRNA levels of ER-stress markers assessed via qPCR. The measurement of enzyme activity of DARS in comparison to control cells is currently ongoing. Oligodendrocytes differentiated from DARS hiPSCs are subjected to morphological characterisation in terms of specific cell markers such as O1, MBP, MOG and the HBSL specific cellular DARS protein complex. Consecutively, a systematic co-culture approach combining HBSL and control neurons, oligodendrocytes and astrocytes in engineered microfluidic devices enables the investigation of myelinating ability as a function of mutation locus in DARS and subsequently severity and onset of HBSL. This project will provide comprehensive molecular insights into the defects in HBSL oligodendrocytes and permit pre-clinical testing of therapeutics for this currently untreatable childhood leukodystrophy. This type of precision medicine provides a paradigm for treating other childhood leukodystrophies and guide therapeutic avenues for other ARS deficiencies.

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W-2083

AN EMPIRICAL LANDSCAPE OF CAS9-MEDIATED GENOME EDITING IN HUMAN PLURIPOTENT STEM CELLS

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CRISPR-Cas9 facilitates genome editing across a wide range of cell types and organisms. Human pluripotent stem cells (hPSCs) are particularly promising for studies of basic and disease mechanisms, but present unique challenges for the scaling of this technology. Here, we investigate CRISPR-Cas9-mediated genome editing in hPSCs at unprecedented scale by applying a standardized editing approach to 58 distinct genes implicated in psychiatric disease. We also present a novel computational tool for resolving the effects of CRISPR-Cas9 editing through targeted sequencing. While efficiency of indel generation varied, this was not correlated with transcription state or nucleosome positioning around target loci. Although the majority of genes proved amenable to editing, we detected variability in indel generation and genomic stability between different hPSC lines, underscoring the need for careful cell line selection and unbiased assessments of genomic integrity.

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W-2085

USING IPSCS TO DELINEATE THE MOLECULAR MECHANISMS CAUSAL TO RAF1-ASSOCIATED HYPERTROPHIC CARDIOMYOPATHY IN NOONAN SYNDROME

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Noonan Syndrome (NS), an autosomal dominant RASopathy disorder, is caused by germ-line mutations that affect the canonical RAS ERK1/2-MAPK pathway. Moreover, >95% of NS patients with an S257L/+ mutation in Raf1 exhibit severe hypertrophic cardiomyopathy (HCM). However, the molecular mechanisms that elicit HCM in these patients remain unknown. Here, we modeled NS-associated HCM by differentiating iPSCs generated from a patient with a Raf1 S257L/+ mutation into cardiomyocytes (iCMs). In addition, we corrected the Raf1 mutation using CRISPR-Cas9 double nickase technology to generate an isogenic control iPSC line. We found that, though proliferation rates were similar (72.25% Ki67+ iCMs ± 4.70 vs 72.50% Ki67+ iCMs ± 2.53, n=4, $p < 0.01$), S257L/+ iCMs displayed increased cell surface area, as compared to isogenic control cells (3,742 μm2 ± 212 vs 2,199 μm2 ± 178, n=6, $p < 0.01$). In addition, S257L/+ iCMs exhibited significant myofibrillar disarray and increased mRNA levels of hypertrophic markers (ANP, BNP or TUBB2B), demonstrating that we could reproduce the HCM phenotype exhibited by NS RAF1 patients. At the molecular level, although S257L/+ iCMs had elevated RAF1 activity, as demonstrated by the robust increase in phosphorylation of its downstream effector, MEK1/2 (5 fold over control level, $p < 0.01$), ERK1/2 itself was only modestly enhanced (1.5 fold over control level, $p < 0.01$). To test whether enhanced ERK1/2 activity was responsible for the increased area of S257L/+ iCMs, we inhibited MEK1/2 or ERK1/2 activity with small molecules or by overexpressing the specific ERK1/2 phosphatase MKP3. Surprisingly, none of these interventions reduced mutant iCMs area, indicating that ERK1/2 was not required for S257L/+-associated hypertrophy. Finally, using RNA sequencing and Ingenuity pathway analysis, we have identified previously unrecognized pathways to be involved in NS-associated RAF1 hypertrophy. Taken together, we have recapitulated the HCM phenotype observed in NS RAF1 S257L/+ patients using human iPSC-derived cardiomyocyte technology and have found that, despite the aberrant increased RAF1 activity in these cells, NS RAF1 S257L/+ mutation modulates hypertrophy through ERK1/2-independent signaling mechanisms.

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W-2087

PROFILING EPIGENETIC ALTERATIONS DURING NEURAL DIFFERENTIATION OF EMBRYONIC STEM CELLS CARRYING DNMT3A OVERGROWTH SYNDROME MUTATIONS

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DNA methylation is an epigenetic modification that is essential for normal development. DNA methylation is catalyzed by a family of DNA methyltransferases (DNMTs). We have recently profiled the DNA methylome, transcriptome and global occupancy of histone modifications (H3K4me1, H3K4me3, H3K27me3, and H3K27ac) in a series of mouse embryonic stem cells (mESCs) with variable DNA methylation levels. We found that genome-wide demethylation alters histone modification patterns in both promoters and enhancer regions, which can be reversed by re-constitution of DNA methylation. Our results support the notion that DNA methylation influences gene expression pattern either directly or via its instructive role for specific histone modifications in regulatory regions. Recently, mutations in the de novo methyltransferase DNMT3A have been found to cause an overgrowth syndrome, characterized by tall stature, macrocephaly, and intellectual disability. To understand how mutations in DNMT3A result in intellectual disability in patients, we have generated a series of isogenic mouse ESC lines containing several mutations identified in overgrowth syndrome patients. We further conducted neural differentiation to study how DNMT3A mutants may lead to epigenetic alterations and affect neural cell differentiation in vitro. We have profiled the transcriptome, methylome, and global histone landscape in neural progenitors converted from mouse control and mutant ESCs carrying DNMT3A mutations. Furthermore, we are currently making isogenic human control and DNMT3A mutant ESCs to determine whether epigenetic alternations in murine cells are conserved in human cell system. Taken together, our study would shed light on the cellular mechanism of human overgrowth syndrome via stem cell differentiation models in vitro.

Funding Source: NIH and California Institute for Regenerative Medicine

W-2089

DISTINCT GENE EXPRESSION CHANGES IN INTESTINAL AND NEURAL ORGANOIDS FROM FAMILIAL PARKINSON'S DISEASE PATIENT-DERIVED IPSCS

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The leucine-rich repeat kinase 2 G2019S (LRRK2GS) mutation is the most common genetic cause of Parkinson's disease (PD). Genome-wide association studies have shown that PD is not only a neurodegenerative disease but also a gastrointestinal disorder. However, its pathogenesis remains unclear. Thus, we developed neural and intestinal tissue models of PD patient harboring the LRRK2GS mutation and investigated the gene expression signatures. We generated induced pluripotent stem cells (iPSCs) from several patient cells carrying the LRRK2GS mutation and from normal individuals. The iPSCs were differentiated into three-dimensional (3D) neuroectodermal sphere (hNES) and intestinal organoid (hIO). To unravel the gene and signaling networks associated with LRRK2GS, differentially expressed genes in the microarray data were analyzed by functional clustering, gene ontology (GO), and pathway analyses. We found that the expression profiles of LRRK2GS were distinct from those of normal control and there are far more differentially expressed genes in the hIO than in the hNES. We suggest that these 3D hNES and hIO models from the same iPSCs of PD patient could be invaluable resources for understanding PD pathophysiology in intestinal as well as neural tissues.

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W-2091

FAMILIAL HYPERCHOLESTEROLEMIA IPSC-DERIVED HEPATOCYTES ENHANCE LDL-C CLEARANCE AND RESPOND TO LDL-C LOWERING DRUGS IN LDLR-/-/RAG2-/-/IL2RG-/- MICE

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Familial hypercholesterolemia (FH) causes elevation of low-density lipoprotein cholesterol (LDL-C) in blood and has increased risk of early-onset cardiovascular disease. We have generated a comprehensive FH stem cell model with differentiated hepatocytes (iHeps) from human induced pluripotent stem cells (iPSCs), including LDLR+/- and LDLR-/- iPSCs generated by zinc-finger nucleases and also LDLR+/- patient-specific iPSCs, for testing novel therapies for FH. We engrafted FH iHeps into Ldlr-/-/Rag2-/-/Il2rg-/- (LRG) mice liver, and assessed the effects of the engrafted iHeps, and their response to statins and PCSK9 antibodies (alirocumab), on LDL-C clearance and endothelial function in-vivo. Our results showed that, the engraftment efficiency of iHeps was around 5-10%, which was evaluated by staining for human albumin in chimeric mice livers. Moreover, the engrafted wild-type and -to a lesser extent- the heterozygous FH iHeps could reduce plasma LDL-C in LRG mice fed with high-fat and high-cholesterol diet. We also observed that the engrafted FH iHeps were responsive to treatment with simvastatin or alirocumab, both of which reduced plasma LDL-C level and improved mouse endothelial function. Although alirocumab was more potent than statins in this model, it required an intact LDLR allele. Thus, our model recapitulates clinical observations of higher potency of alirocumab compared with statins for reversing the consequences of FH, demonstrating the utility for preclinical testing of new therapies for FH patients in particular and in general patients with dyslipidemia.

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W-2093

SYSTEMATIC CELLULAR DISEASE MODELS REVEAL SYNERGISTIC INTERACTION OF TRISOMY 21 AND GATA1 MUTATIONS IN HEMATOPOIETIC ABNORMALITIES

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Transient myeloproliferative disorder (TMD) is a unique hematologic abnormality in infants with Down syndrome (DS). Constitutional trisomy 21 and mutated GATA1, which leads to a truncated protein (GATA1 short; GATA1s), have been considered to be necessary and sufficient for the generation of TMD. Although several studies have shown each role of trisomy 21 and mutated GATA1 in early hematopoiesis in vivo and in vitro, synergistic effects of an additional copy of chromosome 21 and GATA1s remain elusive due to the lack of appropriate disease models. To better understand functional interplay between trisomy 21 and GATA1 mutations in hematopoiesis, we constructed cellular disease models using human induced pluripotent stem cells (hiPSCs) and genome editing technologies, and chromosome engineering techniques. Twenty types of hiPSCs were generated and subjected to hematopoietic differentiation. Constitutive trisomy 21 not only accelerated the production of hematopoietic progenitors (%CD43+), which led to enhanced multilineage differentiation, but also upregulated GATA1s expression, giving rise to the excessive generation of abnormal megakaryoblasts (CD34+CD41+). We also succeeded in isolating an ~4-Mb region critical for hematopoietic defects in DS and identified RUNX1, ERG, and ETS2 as key molecules involved in an interconnected regulatory network. Our study provides insight into the genetic synergy that contributes to multi-step leukemogenesis.

W-2095

MODELING DRUG RESPONSE FOR AUTISM USING IPSC

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Autism spectrum disorders (ASD) are complex neurodevelopmental diseases that affect about 1% of children in the United States. Individuals with ASD are characterized by deficits in verbal communication, impaired social interaction and present limited and repetitive interests and behavior. The major impediment to testing hypotheses and potential therapeutic interventions for autism is the lack of relevant animal and cell models. Reprogramming of human somatic cells to induced pluripotent stem cells (iPSC) provided an exciting opportunity to produce a relevant human cellular model for complex neurogenetic disorders such as ASD. Here we use Multielectrode Arrays to perform functional field potential analysis of iPSC-derived neuronal populations from ASD individuals and controls during development and after treatment with a drug that is currently in clinical trials for ASD (Insulin growth factor 1, IGF1). Our preliminary results indicate that ASD neurons respond to drug treatment by increasing neuronal spiking and neuronal bursts. Additionally, we performed expression profile analysis on developing ASD neurons and neurotypical controls after drug treatment to uncover pathways that are potentially involved in the recovery of the neuronal activity. Our data indicates that IGF1 has a specific molecular effect on ASD neurons. Studying biological basis of ASD and cellular drug responsiveness would likely lead to the development of clinically useful biomarkers of risk for this disorder, which may lead to the development of novel therapies.

W-2097

DIS3L2-MEDIATED SURVEILLANCE OF RIBOSOMAL RNAS SAFEGUARDS PROTEIN TRANSLATION AND CELL DIFFERENTIATION

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Stem cells are emerging as valuable tools for modeling diseases. Here, we use mouse embryonic stem cells (ESCs) to unravel the mechanisms involved in Perlman overgrowth syndrome and Wilms tumor susceptibility caused by mutations in DIS3L2. DIS3L2 is a 3'-5' exoribonuclease and is involved in degradation of 3'-oligo uridylated RNA substrates. Recently, we identified a DIS3L2-mediated decay (DMD) pathway for certain noncoding RNAs, including Rmrp, 7SL, U1 and U2 snRNAs, as well as 5S ribosomal RNAs (rRNAs) and other long noncoding RNAs. Now, we systematically analyze the effect of DIS3L2 loss on rRNA biogenesis intermediates. We find that upon DIS3L2 depletion, and downstream of nuclear exosome function, a cytoplasmic 3'-uridylated 5.8S rRNA intermediate with a 13 nucleotide

extension accumulates. This intermediate, which we name pre-6S rRNA, is incorporated into translating polyribosomes in DIS3L2-deficient cells and impairs homeostatic protein translation. While undifferentiated cells appear unaffected by DIS3L2-deficiency with obvious no defects in self-renewal or expression of pluriptency markers, DIS3L2-depleted ESCs manifest overgrowth and promiscuous protein translation upon kidney-directed or spontaneous embryoid body (EB) differentiation, consistent with the features of Perlman syndrome . We will discuss how our ESC-based model helps understand the etiology of Perlman syndrome and Wilms tumor.

W-2099

DOSAGE COMPENSATING TRISOMY 21 IN DS IPSCS MITIGATES ABNORMALITIES ASSOCIATED WITH HEMATOPOIETIC DIFFERENTIATION

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Down syndrome (DS) patients have higher risks of multiple pathologies including hematopoietic abnormalities. Overproduction of hematopoietic cells in fetal liver predisposes patients to myeloproliferative disorder and acute megakaryocytic leukemia. We previously showed the ability to dosage-compensate chromosome 21 genes in DS iPSCs by targeted insertion and expression of XIST, which encodes a long non-coding RNA that spreads and silences its parent chromosome in cis. Here, we test and demonstrate the feasibility that DS associated hematopoietic abnormalities can be prevented by XIST-mediated chromosome 21 silencing during hematopoietic differentiation of transgenic DS iPSCs. Additionally, analysis of different steps in hematopoietic differentiation indicates that trisomy 21 enhances the differentiation of hematopoietic progenitors from hemogenic endothelium. Results further suggest involvement of non-chromosome 21 genes in trisomy 21 associated overproduction of early hematopoietic progenitors. This study demonstrates a proof-of-principle for the potential application of this novel approach to both mitigate and understand underlying pathologies of trisomy 21.

W-2101

CO-EXISTENCE OF R-LOOPS WITH SINGLE STRAND DNA SLIP-OUTS ACROSS THE CGG REPEATS IN FRAGILE X AFFECTED PLURIPOTENT STEM CELLS WITH AN UNMETHYLATED FULL EXPANSION

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Fragile X syndrome (FXS) is the most common non-chromosomal form of heritable mental retardation. It results from a CGG repeat expansion in the 5′-UTR of the X-linked FMR1 gene. When the CGGs exceed 200 copies (full mutation) they lead to CpG methylation and epigenetic gene silencing of FMR1 in a developmentally regulated process. In addition, when the CGGs expand, they become increasingly unstable, leading to high degree of variation in expansion size between and within tissues of affected individuals (repeat somatic instability). Our research focuses on when and how FMR1 gains aberrant methylation, and whether hypermethylation is mechanistically associated with CGG somatic instability. Taking advantage of a large set of FXS HESC lines (9 in total) and patient-derived iPS cells, we demonstrate that CGG instability exists in these cells, and is tightly correlated with the methylation status and transcriptional activity of FMR1. Furthermore, we provide evidence for the existence of persistent DNA:RNA hybrids (R-Loops) across the CGG repeats in FXS HESCs/iPSCs with an active FMR1, and demonstrate their co-existence with single strand DNA displacements from the G-rich non-template DNA by finely mapping their 5’ and 3’ boundaries. In accordance with our findings, we put forward a model that relies on R-loop formation to explain how epigenetics and gene transcription are mechanistically associated with CGG somatic instability in fragile X syndrome.

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W-2103

NEURODEVELOPMENTAL ABERRATIONS IN AUTISM SPECTRUM DISORDERS ARE CAUSED BY INTRINSIC DYSREGULATION OF TIME-CRITICAL GENE NETWORKS

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Autism spectrum disorder (ASD) is a disorder of early brain development. One of the current challenges in understanding ASD pathophysiology is to determine critical neurodevelopmental periods and cellular states that might provide the ground for disease propensity. Here we use a longitudinal approach to investigate neurodevelopmental alterations in individuals with severe idiopathic ASD by employing induced pluripotent stem cell (iPSC)-based model systems that recapitulate fetal cortical development. Longitudinal reconstruction of the transcriptome revealed altered expression dynamics of genes involved in neuronal differentiation, cell morphogenesis and distinct pathways with critical temporal features during development. Using gene network analysis in combination with dynamic time warping, we identified three common time-related modules with high enrichment for autism risk factors. ASD-derived neurons exhibited an early structural growth acceleration with premature initiation of the neuronal program corresponding to a specific neurodevelopmental module, whose temporal progression appeared markedly accelerated in ASD neurons. Temporal reconstruction of this module revealed permissive neurodevelopmental signatures in ASD progenitors with insufficient suppression of network hubs. To evaluate our findings, we showed that circumventing the neural progenitor stage by using direct conversion of iPSCs into induced neurons (iPSC-iN) restored the altered neurodevelopmental trajectory in ASD neurons. These results show that developmental growth rates and structural development of cortical neurons are partly determined early in the linage and identify intrinsic differences in neural progenitor cells as developmental precursor for ASD propensity.

W-2105

ZIKA VIRUS ALTERS DNA METHYLATION PATTERN OF NEURAL GENES

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Zika virus (ZIKV) infection in early pregnancy is known to cause microcephaly at birth, but it remains unclear whether it can cause neurological sequelae later in life. We hypothesized that the virus might cause epigenetic changes and examined its effects on DNA methylation in human neural development by performing DNA methylation profiling of ZIKV-infected human embryonic stem cell-derived cerebral organoids and neuronal cells. We found that ZIKV infection alters the DNA methylation landscape of neural progenitor cells, astrocytes and terminally differentiated neurons. Many of the genes most prominently affected have been implicated in neural disorders such as mental retardation and schizophrenia. These results suggest that ZIKV infection of the nervous system could lead to myriad post-infectious neuropsychiatric complications.

W-2107

CEREBELLAR DIFFERENTIATION AND MATURATION FROM HUMAM INDUCED PLURIPOTENT STEM CELLS

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Human induced pluripotent stem (iPS) cells have great potential for disease modeling. However, generating iPS cell-derived models to study brain diseases remains a challenge. In particular, our ability to differentiate cerebellar neurons from pluripotent stem cells is still limited (Muguruma et al. 2015). Here we describe the long-term culture of cerebellar neuroepithelium formed from human iPS cells, recapitulating the early developmental events of the cerebellum. Pluripotent cells were differentiated into cerebellar organoids in

a 3D culture system by sequential addition of growth factors, including FGF2, FGF19 and SDF1. We observed the formation of a multilayered neuroepithelial structure with well-defined apico-basal polarity of distinct cerebellar layers consisting in Rhombic-lip-derivative (BARHL1+), Purkinje cells precursors (Olig2+ and SKOR2+) and Ventricular zone of progenitors (Sox2+ and N-cad+). Differentiating cells were maintained for up to 145 days using the recently described serum-free neuronal basal medium (Bardy et al. 2015). Distinct types of cerebellar neurons were generated, including Purkinje cells (Calbindin+), Granule cells (BARHL1+ and Pax6+), Golgi cells (Neurogranin+ and GAD65+), Deep cerebellar nuclei projection neurons (TBR1+) and Non-Golgi-type interneurons (Parvalbumin+ and Calbindin-). When organoids were dispersed and cells replated on a laminin-coated surface, intricate networks of matured (MAP2+) neurons formed. In conclusion, we have established for the first time a methodology to differentiate cerebellar neuroepithelium from iPS cells using defined serum- and feeder-free culture conditions. We believe this work sets the foundation to develop new models to study neurodegenative diseases such as ataxias that are caused by dysfunction of the cerebellum.

W-2109

HUNTINGTON'S DISEASE PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS AND NEURAL STEM CELLS: INSIGHTS INTO EARLY METABOLIC AND MITOCHONDRIAL DEFECTS

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Huntington's disease (HD) is an autosomal dominant disease caused by an expansion of CAG repeats in the HTT gene encoding for mutant huntingtin (mHTT). Selective loss of striatal medium spiny neurons is a major hallmark of HD, with symptoms ranging from psychiatric disturbances, involuntary movements and cognitive deficits, leading to dementia. mHTT expression alters cellular physiological mechanisms inducing mitochondrial and metabolic dysfunction. An attractive model to study early disease mechanisms are HD patient-derived induced pluripotent stem cells (HD-iPSC). Thus, in this study we aimed to investigate detailed mitochondrial-based mechanisms in HD-iPSC and derived neural stem cells (NSC) versus respective control cells. Mitochondria from HD-iPSC and NSC appear more fragmented with immature round shape morphology linked to decreased levels of OPA1, required for mitochondrial fusion. Additionally, both HD-iPSC and NSC mitochondria exhibited lower basal respiration, decreased ATP/ADP levels, decreased levels of nuclear-encoded complex III subunits and corresponding activity, and were highly dependent on glycolysis, when compared to control cells. This was accompanied by increased levels of mitochondrial reactive oxygen species. Mitochondria from HD cells were also more hyperpolarized due to ATP synthase reversal, and showed enhanced ability to accumulate calcium. HD-iPSC and HD-NSC also displayed increased phosphorylation of pyruvate dehydrogenase (PDH) E1α subunit at Ser232, 293 and 300, reflecting reduced PDH catalytic activity, which was related with increased mRNA levels of PDH kinase 1 and reduced mRNA levels of PDP1 (PDH E1α phosphatase), observed in HD-iPSC. In conclusion, HD-iPSC and HD-NSC are more glycolytic, supported by diminished PDH activity, reduced complex III activity and decreased oxidative phosphorylation, resulting in reduced ATP generation. These data reinforce metabolic and mitochondrial dysfunction as early events in the HD pathogenic cascade.

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W-2111

EFFICIENT DIFFERENTIATION OF HUMAN ASTROCYTES FROM IPS CELLS - APPLICATIONS FOR SCHIZOPHRENIA STUDIES

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Schizophrenia (SZ) is a devastating mental disorder with a prevalence of 1 % worldwide. Although the etiology of

SZ still remains unclear, the changes in both genetic and environmental factors already in the first trimester of pregnancy can lead to the activation of pathologic neural circuits at young adulthood and to the appearance of positive and negative symptoms. Twin studies are a vital resource for understanding genetic contribution to the etiology of SZ. The concordance rate for monozygotic twins is only 41-65%, even though sharing the same genome and childhood environment. While the majority of studies have focused on neuronal pathology and possible perturbations of neuronal development in SZ, astrocytes exert a number of important functions in the brain and there is growing evidence that astrocytes have a contributing role in the pathophysiology of SZ. The aim of this study was to generate a human cell model by differentiating a pure population of astrocytes from iPSC of six monozygotic twin pairs discordant for SZ and healthy controls. Astrocytes were differentiated from iPSC via neuroepithelial interphase. Rosette-like structures were picked up and cultured as spheres for up to 5 months before maturation in the presence of CNTF and BMP4. Cells were characterized based on morphology, RNA and protein expression. The cells were positive for GFAP (60 %), Vimentin (80 %) and S100b (100 %). Up to 85% of the cells were able to uptake glucose. Inflammatory stimulation of astrocytes by TNFα and IL-1β but not IFNγ resulted in release of IL-6 inducing reactive astrocytic phenotype. Regulation of Ca2+ release from the astrocyte ER showed statistically significant difference not only between the healthy controls and twin pairs, but also between the twins from pairs discordant for SZ. The differences between the groups will be investigated by the whole-transcriptome analysis in order to identify the key mechanisms responsible for the phenotype of SZ. Taken together, this model offers a valuable tool for studying the disease mechanisms of schizophrenia as well as in vitro testing of drugs.

W-2113

MODELING CYSTIC FIBROSIS FOR DRUG-PROFILING ON HEPATIC-BILIARY ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

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Cystic Fibrosis (CF) associated liver disease (CFLD) is the third most common death in CF patients. In the liver, the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel protein (conducting chloride and bicarbonate ion) is anchored in the apical membrane of cholangiocytes (bile duct epithelial cells), where it regulates the flux of electrolytes and water. Abnormal CFTR protein function in CF patients leads to impaired secretion and deposition of viscous, acidic bile resulting in damage to the liver. In this study, we have used human pluripotent stem cells (hPSCs) to model human biliary duct development and CFLD in vitro, building on our previous studies that showed that NOTCH signaling promotes the specification of cholangiocytes from hepatic progenitors known as hepatoblasts (Ogawa et al. Development, 2013, Ogawa et al. Nat. Biotechnol. 2015). To induce Notch signaling in our current studies, we cultured hPSC-derived hepatoblasts as aggregates with OP9 stromal cells engineered to express Jagged1. Within 2 weeks of culture, these chimeric aggregates formed cysts that expressed CFTR and contained primary cilia, characteristics of cholangiocytes. These cysts displayed a forskolin-induced CFTR mediated swelling response, indicative of cholangiocyte function. CF patient iPSC-derived organoids, showed a deficiency in the swelling response that was partially rescued by small molecule CFTR modulators (VX809+VX770), currently being used in the clinic. When plated on a matrigel substrate, the cysts generate a monolayer of cholangiocytes that maintain expression of CFTR. To analyze CFTR function in these cells, we used an apical chloride conductance assay that measures channel activity through changes in membrane potential detected in a plate reader. Using this assay, we are currently profiling drug responses in iPSC-derived cholangiocytes generated from patients carrying different CFTR mutations (F508del and p.Ile1234_Arg1239del), enabling the development of patient and tissue specific therapies for this disease.

REPROGRAMMING

W-2115

EVIDENCE OF CLINICAL SIGNIFICANCE OF MONOCYTE/MACROPHAGES IN OSTEOARTHRITIS AND THE EFFECT OF OSTEOARTHRITIS MICROENVIRONMENT ON MONOCYTE/MACROPHAGE FUNCTIONALITY

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Osteoarthritis (OA) is a progressive and debilitating joint disease with high prevalence in an ageing demographic. Inflammatory cells, particularly monocytes/macrophages (MΦ) are present in the OA synovium and involved in its progression. Modification of local MΦs may thus be of therapeutic value. However, it is unknown whether MΦs have clinical significance in OA, even less is known about how the OA microenvironment modulates the MΦs. Synovial fluid (SF) from OA knees

were acquired during arthroscopy (defined here as early OA) and arthroplasty (late OA). Patients completed standard questionnaires to determine their Knee injury and Osteoarthritis Outcome Score (KOOS). Monocytes were isolated from blood of healthy donors. MΦs were functionally polarized towards M2 (IL10+TGFβ) and M1 (IFNγ+LPS) or left in medium alone (M0) for 2 days and later exposed to OA SFs for 7 days. MΦs were characterized by flow cytometry. The characterization of monocytes in SFs, indicated that levels of pro-inflammatory subsets inversely correlate with patient's KOOS quality of life. Ex-vivo polarized MΦs displayed a clear M1 and M2 phenotype, with high levels of HLADR and CD86, low levels of CD163 and endocytosis for M1-MΦs; and a contrasting phenotype and functionality for M2-MΦs. Upon stimulation with OA SFs for 7 days, there was an upregulation of CD163 and CD206 expression in all MΦs, but to a greater extent in early OA SFs where they remained higher in M2-MΦs. This was mirrored by greater inhibition of T helper cell proliferation by M2 and M0-MΦs in early OA SFs. Endocytosis was upregulated in both early and late OA SFs, but M2-MΦs retained the highest endocytic capacity. Interestingly, both early and late OA SFs also upregulated CD86 (an M1 marker) in all MΦs. HLADR, another M1 marker, had greater upregulation in late OA SFs on M2 and M0-MΦs with no changes in M1-MΦs. Taken together pro-inflammatory MΦs appear to have clinical relevance in OA. Our preliminary data indicates that the early OA microenvironment is more conducive to maintaining the M2-MΦs phenotype and functionality vs. the late OA microenvironment. This suggests that early OA patients might be more promising candidates for the therapeutic use of ex-vivo polarized M2-MΦs.

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W-2117

NON-VIRAL INDUCTION OF TRANSIENT CELL REPROGRAMMING IN MOUSE SKELETAL MUSCLE TO ENHANCE TISSUE REGENERATION

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Somatic cells can be reprogrammed to pluripotency in vivo by overexpression of defined transcription factors. While their sustained expression triggers tumorigenesis, transient reprogramming induces pluripotency-like featuresand proliferation only temporarily, without teratoma formation. We sought to achieve transient reprogramming within mouse skeletal muscle with a localized injection of plasmid DNA (pDNA) and hypothesized that this would enhance regeneration after severe injury. Intramuscular administration of reprogramming pDNA rapidly upregulated pluripotency (Nanog, Ecat1, Rex1) and early myogenesis genes (Pax3) in the healthy gastrocnemius of various mouse strains.Mononucleated cells expressing such markers appeared promptly in clusters among myofibers, but proliferated only transiently and did not lead to the generation of teratomas. Nanog was also upregulated in the gastrocnemius when reprogramming factors were administered 7 days after laceration of its medial head. Enhanced tissue regeneration after reprogramming was manifested by the accelerated appearance of centro-nucleated myofibers and reduced fibrosis. These results suggest that in vivo transient reprogramming may constitute a novel strategy towards the acceleration of regeneration following muscle injury, based on the induction of transiently-proliferative, pluripotent-like cells in situ. Further research to achieve clinically meaningful functional regeneration is warranted.

W-2119

HIGHLY EFFICIENT ONE-STEP GENERATION OF PARKINSON'S DISEASE PATIENT DERIVED INDUCED NEURONS USING A NOVEL VECTOR SYSTEM

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Direct conversion of adult human fibroblasts into mature and functional neurons, termed induced neurons (iNs) was achieved for the first time five years ago. This technology offers a shortcut for obtaining patient and disease specific neurons for disease modeling, drug screening and other biomedical applications. Despite their great promise, reprogramming roadblocks have prevented the generation of iNs at a sufficiently high yield from adult dermal fibroblasts, which has significantly limited the adoption of this technology. To overcome this, we have developed a new highly efficient dual promoter-based vector system that results in efficient co-delivery of the two reprogramming factors Brn2a and Ascl1 in combination with either neuron specific microRNAs or the inhibition of the RE1-silencing transcription factor (REST). Global gene expression analysis showed that while both strategies resulted

in induction of a neuronal program and similar level of neural conversion, the inhibition of REST induces the expression of additional genes that are related to neuronal identity and function. Based in this, we developed an optimized one-step method to efficiently reprogram dermal fibroblasts from elderly individuals using a single vector system and demonstrate that it is possible to obtain iNs of high yield and purity from aged individuals, including Parkinson's disease patients. We are now evaluating the conversion capability of these skin fibroblasts and our preliminary results suggest that iN cells from sporadic PD patients reprogram in a similar fashion as that of healthy individuals and could thus serve as a tool to model intracellular pathological features associated with PD.

W-2121

ACTIVATION OF THE TRANSCRIPTION FACTOR SRF DESTABILIZES CELLULAR IDENTITY BY SUPPRESSING CELL-TYPE-SPECIFIC GENE EXPRESSION PROGRAMS

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Multicellular organisms consist of multiple cell types, of which identity is stably maintained to secure organismal homeostasis. The identity of each cell type is primarily maintained by cell-type-specific gene expression programs, but mechanisms that suppress these programs are poorly defined. Here we show that serum response factor (Srf), a transcription factor that is activated by various extracellular stimuli, can repress cell-type-specific genes and promote dedifferentiation. Manipulations that decrease β-actin monomer resulted in the activation of Srf, which downregulated cell-type-specific genes and altered epigenetics in enhancers and chromatin organization. Mice overexpressing Srf exhibited various pathologies, which had been associated with single nucleotide polymorphisms on cell-type-specific enhancers. Our results demonstrate an unexpected function of Srf via a mechanism by which extracellular stimuli actively destabilize cell identity and suggest Srf involvement in a wide range of diseases.

W-2123

EFFECT OF REPLACEMENT OF C-MYC WITH L-MYC ON SENDAI-BASED REPROGRAMMING METHOD

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As induced pluripotent stem cells (iPSCs) move toward clinical applications, it is necessary to minimize oncogenic risk in order to ensure safe transplantation of the cells. Previously used reprogramming protocols utilize the transcription factor c-Myc that is a known proto-oncogene associated with different cancers. Another transcription factor in the Myc family, L-Myc has been found to have low transformation activity and is less frequently observed in human cancers. The aim of this study is to compare the reprogramming efficiencies of c-Myc and L-Myc in a variety of different blood cell types. Peripheral blood mononuclear cells (PBMCs), CD34+ cells, and T-cells were cultured using a xeno-free workflow and reprogrammed using both a Sendai virus containing c-Myc and L-Myc. The number of AP+ colonies and relative reprogramming efficiencies were determined by terminal alkaline phosphatase staining. Statistical data analysis was carried out using JMP. Results indicate that although for some cell types L-Myc yielded lower reprogramming efficiencies, the colonies obtained had the same morphology as c-Myc colonies. The ability of L-Myc to reprogram blood cells into iPSCs while reducing the oncogenic risk makes it a good candidate for the generation of clinical-grade iPSCs.

Funding Source: This research was funded by California Institute for Regenerative Medicine and Thermo Fisher Scientific.

W-2125

MAPPING DEDIFFERENTIATION IN VIVO

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The manipulation of cell fates through reprogramming is one of the most exciting advances in recent years. The most important breakthrough in the field occurred when Yamanaka first illustrated the possibility to convert differentiated cells into pluripotent stem cells by the ectopic expression of 4 transcription factors, Oct4, Sox2, Klf4 and cMyc (4F). Our laboratory has demonstrated that transient expression of these 4 factors leads to teratoma formation in mice, indicative of in vivo reprogramming. We are interested in

deciphering de-differentiation induced by the 4F in vivo. Using the reprogrammable mice, we test if dedifferentiation recapitulates intermediate phases of embryonic development in reverse. For this purpose, we focused on pancreas which we found to be the organ with the highest reprogramming efficiency in vivo. We found that in vivo reprogramming of pancreas leads to the loss of its acinar identity and the acquisition of an atypical tubular morphology different from ductal cells. Moreover, these atypical tubular cells are able to form organoids in culture in contrast to normal pancreatic acinar cells. Our preliminary data suggests that reprogramming in vivo may generate a type of progenitor cells distinct from those already described during pancreatic development. Taking into account that the presence of adult pancreatic stem cells is still debated, this new aspect of pancreas dynamics could be of great importance for regenerative medicine and especially diabetes.

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W-2127

THE FIRST STEP TO CONSERVING ENDANGERED SPECIES THROUGH STEM CELLS

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Since the development of induced pluripotent stem cells (iPSCs), stem cell research has expanded dramatically, but most of this research focuses on human and mouse models. The first reported generation of endangered species iPSCs was published in 2011 (Nature Methods 8, 829). These iPSC lines, from the drill and the northern white rhinoceros (NWR), were reprogrammed using the integrating lentivirus. To make integration-free iPSCs, we have reprogrammed NWR fibroblast cell lines from several animals using Sendai virus. Fibroblast cell lines were obtained from the San Diego Zoo's Frozen Zoo® repository of biomaterials, which contains cell lines from 12 different NWRs, and captures high genetic variability of this species. These cell lines were established from 3mm biopsy punches and banked over the last 38 years. Fibroblasts were grown in a medium optimized for the growth of rhino cells, which has now also been incorporated into the formulation of a rhino specific stem cell medium (KB medium). Compared to human iPSCs, NWR iPS cells and colonies have distinctive morphologies, which are consistent among lines from this species. Pluripotency and differentiation potential was confirmed with immunocytochemistry markers for pluripotency in the iPSCs and markers for all three germ layers in differentiated embryoid bodies (EBs). qRTPCR and RNAseq analyses confirmed expression of pluripotency-associated genes in the iPSCs, and germ layer-associated genes in the EBs. All fibroblast cell lines and corresponding iPSC lines were found to be karyotypically normal. The NWR is functionally extinct, with only three, non-reproductive, living individuals remaining. These NWR iPSCs are the first step in a plan to rescue this species through assisted reproduction (Zoo Biology. 35: 280, 2016).

W-2129

DETERMINISTIC IPSC REPROGRAMMING PLATFORM BASED HIGH-THROUGHPUT SMALL MOLECULE SCREENING IDENTIFIES NOVEL MODULATORS OF IPSC REPROGRAMMING

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Somatic cells can be reprogrammed into iPSCs the exogenous expression of four transcription factors: Oct4, Sox2, Klf4, c-Myc (OSKM). This process holds a great promise for research and future therapeutic potential, but is currently hard to investigate due to its low efficiency and inherent stochastic nature. Our lab has previously demonstrated that optimized reduction of core component in the NuRD complex (Nucleosome Remodeling Deacetylase), Mbd3 or Gatad2a can dramatically improve the efficiency of reprogramming and to alter its dynamics towards a deterministic and synchronized process (up to 100% efficiency in 8 days). Here I describe the use of such defined and highly efficient systems in order to gain new knowledge on the reprogramming process, its roadblocks and facilitators. These platforms were customized and adapted to reveal novel pathways and players affecting reprogramming via two different high-throughput small molecule screens: 1) The first screen sought to reveal suppressors of iPSCs formation and is conducted using Gatad2a-Mbd3/NuRD-deficient cells. 2) The second screen was designed for identifying reprogramming boosters facilitating a similar phenotypic effect on wild type cells, as seen in NuRD-deficient cells. Top candidates from both screens were identified and validated. The signaling, transcriptional and/or epigenetic pathways influenced by these novel candidates, were investigated using different pathway modifiers and by genetic manipulations, in order to reach a deeper understating of the mechanisms underlying the iPSC reprogramming process.

W-2131

FLUORESCENT TOOLS TO MONITOR THE EFFICIENCY OF NON-INTEGRATIVE SOMATIC CELL REPROGRAMMING

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Reprogramming of somatic cells to human iPSCs is a complex process typically taking 12-15 days before formation of small iPSCs colonies are observed and 25-30 days before colonies are of sufficient size to passage. In most cases, researchers have had to wait the requisite 15-30 days to determine whether their specific reprogramming conditions were effective. We previously developed a synthetic self-replicative RNA (srRNA) that efficiently generated human iPSCs by expressing the reprogramming factors (OKSG and OKSG-cMyc) using the RNA replicon of Venezuelan Equine Encephalitis (VEE) virus. To assess the transfection efficiency of srRNAs in somatic cells that may have differing proliferative capacity, we generated two self-replicating RNAs that express either the green fluorescent protein (TagRFP2) or the red fluorescent protein (TagRFP). We also generated a srRNA that simultaneously express the five reprogramming factors (OKSG-cMyc) along with the TagRFP gene (5F-RFP). In the presence of B18R protein which suppresses the cellular interferon response, self-replicative TagRFP and TagGFP2 RNAs resulted in continuous stable red and green fluorescence, respectively. Removal of B18R protein resulted in rapid degradation of the srRNAs with an accompanying disappearance of the fluorescent proteins. Similarly, the 5F-RFP was able to monitor transfection efficiency and importantly, iPS generation for the first 15 days with observations of small iPS colonies with red fluorescence. 5F-RFP is currently being used to further optimize the kinetics and efficiency of human iPS reprogramming. In summary, new fluorescence tools have been developed to monitor iPS generation. These fluorescently labeled srRNAs are compatible with fluorescent microscopy and flow cytometry and will greatly aid in the optimization of experimental conditions for iPS generation.

TECHNOLOGIES FOR STEM CELL RESEARCH

W-2133

PRECISE GENE EDITING OF HUMAN STEM CELLS USING NOVEL NANOPARTICLES CONTAINING CRISPR NUCLEASES WITH DNA REPAIR TEMPLATES

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The CRISPR-Cas9 system has rapidly revolutionized the genome editing field. However, there are still significant hurdles in generating precise edits in stem cells for various applications in genomic and regenerative medicine as well as drug discovery, disease modeling and toxicology. For in vivo clinical applications, high efficiency editing of stem/progenitor cells can promote regeneration. For in vitro applications, cultured cells are often subjected to many rounds of replication while screening for desired mutants, particularly when multiple genes are targeted. This prolonged period of culture results in the accumulation of undesired mutations throughout the genome. To address these issues we have developed a novel sgRNA that, when combined with Cas9, is capable of forming an all-in-one gene-editing particle that simultaneously delivers all components necessary for precise gene-editing. These particles can be engineered to increase the speed and accuracy in which gene-edited cell lines can be created. In one design of the nanoparticle, we are able to conjugate fluorochromes to specific sgRNA-donor pairs of interest. Through the use of fluorescence activated cell sorting (FACS) we are able to preferentially sort out cells that are accurately edited in a multiplexed manner at up to four targeted loci, with minimal editing or no undesired indel mutations. Use of these new gene-editing materials with human stem cells should advance our abilities to directly write the genome within the lab and within the body.

W-2135

GENERATION AND CHARACTERIZATION OF TEN MOST FREQUENT HLA-HOMOZYGOUS IPSC LINES IN KOREAN POPULATION

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Although induced pluripotent stem cells (iPSCs) can be potentially useful for treating patients without immune rejection, in reality, it will be extremely expensive and labor-intensive to make autologous iPSCs to realize personalized medicine. To overcome these limitations, we have utilized the advantage of Human Leukocyte Antigens (HLA) system, in which HLA-matched cells or tissues can be transplanted into recipients without or with minimal immune rejection. In this study, we screened over 4,000 frozen cord blood samples that have been HLA typed using 4-digit method. Among them, we selected samples homozygous for ten most frequent HLA haplotypes in three loci (i.e., HLA-A, B, and DRB1). Afterwards, we generated and established the corresponding ten iPSC lines using a non-integrating episomal plasmid-based reprogramming method under feeder-free and xeno-free conditions. Next, we performed a comprehensive analysis in order to characterize the cell lines, which include morphology, expression of pluripotent markers and cell surface antigens, three-germ layer formation, vector clearance, mycoplasma/microbiological contamination, endotoxin and short tandem repeat (STR). We also conducted various genomic analyses using the microarray and comparative genomic hybridization (aCGH)-based single nucleotide polymorphism (SNP) and the copy number variation (CNV) to confirm whether these established cell lines are genetically stable following iPSC generation and the subsequent culture period. In theory, these ten HLA-homozygous iPSC lines can cover 41.2% of Korean population. According to comparative HLA type analysis, these Korean iPSC lines are not only useful for Korean population but also useful for diverse Asian populations, including Japan. Taken together, these results strongly suggest that these ten HLA-homozygous iPSC lines that we have established largely meet the criteria required for clinical-grade cells, which will serve as an important basis for developing clinical-grade iPSC lines in the future.

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W-2137

CREATING AN OPEN SOURCE COLLECTION OF GFP-TAGGED HUMAN IPSC LINES TO MODEL STEM CELL ORGANIZATION AND DYNAMICS

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The Allen Institute for Cell Science (AICS) is creating a dynamic visual model of hiPSC organization to aid in understanding and predicting normal and pathological cell states. Our approach utilizes CRISPR/Cas9 gene editing to introduce fluorescent tags via homology driven repair (HDR) into genomic loci whose products localize to specific organelles. Editing yields isogenic hiPSC lines expressing fusion proteins unique to each cell line under endogenous regulation. Live cell imaging, image analysis and modeling, and open distribution to the scientific community of each unique cell line defines our endeavor. Because we will perform systematic editing at numerous genomic loci, our data has begun and will continue to elucidate variables and trends important for gene editing in stem cells. Here we present our CRISPCR/Cas9- based gene editing protocol and workflow to introduce fluorescent tags into the genome of stem cells and our initial progress and conclusions from the generation of ~1000 clones spanning 10 different targets. We will describe our screening strategy to identify clones harboring precisely incorporated GFP tags at the genomic loci and demonstrate the various consequences of imprecise editing. We will also present our quality control assays including the characterization of stem cell properties, off-target analysis, karyotyping, directed differentiation into cardiomyocytes, and next generation sequencing. Furthermore, we will present data supporting the correct subcellular localization of the tagged proteins from imaging studies. In experiments initiated to date we have generated hiPSC lines for ~15 major cellular structures including cell-matrix adhesions, the actin and microtubule cytoskeleton, mitochondria, desmosomes, endoplasmic reticulum, and nuclear envelope.

W-2139

INCORPORATING AUTO- AND PARACRINE SIGNALING TO OPTIMIZE HUMAN STEM CELL DIFFERENTIATION EFFICIENCY: A VERSATILE MICROFLUIDIC CELL CULTURE PLATFORM

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We present a versatile microfluidic platform capable of efficient optimization of differentiation conditions for pluripotent stem cells (PSCs). The device has three main advantages: ease of manufacture, minimization of manual labor by passive, on-chip dilution and mixing, and a large surface area to volume ratio in on-chip culture wells, permitting study of auto/paracrine signaling. Wells are kept isolated from diffusive contact, allowing for controlled culture with or without continuous perfusion. Microfluidic devices are typically created using photolithography to produce a silicon wafer mold on which to cast polydimethylsiloxane (PDMS), a labor intensive process requiring a cleanroom facility that creates a cost barrier for researchers not associated with a dedicated microfluidics group. By designing a mold that can be 3D printed, we bypass the need for special facilities and incur dramatic cost savings. Although process optimization is most efficiently achieved through iterative factorial design, the number of experimental conditions that must be setup can be limiting. To address this problem and improve reproducibility, the device automatically performs combinatorial mixing, dilutions, and distribution for a 3 factor experimental design with center points. Notably, this is accomplished passively by the device without the need for pneumatic control. Using multiple devices in parallel, the number of experimental factors can be increased and include variables such as time spent in differentiation medium or, indirectly, endogenous factor accumulation through medium exchange frequency. Accumulation of endogenous factors has been shown to significantly affect cell survival and differentiation efficiency, however relevant concentrations are largely unattainable in traditional culture plates and flasks. We demonstrate device utility by optimizing differentiation medium composition for a recently published protocol for the differentiation of human PSCs toward auditory neurons. In addition to medium optimization, our device has the potential to be used for rapid optimization of other processes including antibody concentration in immunocytochemistry, reagent concentration for lipofection protocols, siRNA delivery protocols, in vitro testing for combination drug effects, and more.

Funding Source: NIH (NIDCD (K08 Clinician Scientist Award)); American College of Surgeons Clinician Scientist Grant; Trilogical Society Clinician Scientist Grant

W-2141

EARLY RESULTS: HIGH SENSITIVITY MAGNETIC PARTICLE IMAGING SYSTEM FOR STEM CELL RESEARCH

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Magnetic Particle Imaging (MPI) is an emerging cellular imaging technique that can non-invasively detect iron oxide (IO) labeled stem cells in vivo. The technique does not use ionizing radiation and can be scaled to humans. Stem cells can be loaded with IOs by co-incubation, without negatively impacting phenotype or viability. MPI images have an unambiguous contrast that shows both the concentration and position of a tracer, enabling linear quantitation of cell number at any location (2). The spatial resolution is on the order of MRI, and MPI ease of use echoes Optical imaging. In this study, we optimized the resolution, signal linearity, and sensitivity of a field-free line (FFL) MPI scanner. Phantoms were prepared from commercial nanoparticles (VivoTrax, Magnetic Insight Inc, CA) and MPI tailored IOs. Imaging was performed with the MOMENTUM MPI system (Magnetic Insight Inc., CA). Data analysis was performed in VivoQuant (inviCRO, MA). To measure spatial resolution, two 1µL point sources were imaged at various distances. At 600 µm separation, both point sources can still be distinguished, suggesting better than 400 µm system resolution. To measure signal linearity and sensitivity, stock solutions were serially diluted with deionized water to 550 pg Fe/µL- 5.5 µg Fe/µL. The MPI tailored IOs were found to display 3-fold more signal then VivoTrax, in agreement with previously published work (Ferguson 2015). The linear signal range extends over 4 orders of magnitude with exceptional linearity (R^2 = 0.99). Images of both a 100 µL sample containing 550 pg Fe/µL and of a 1 µL point source containing 1100 pg Fe showed good signal-to-noise ratio, demonstrating a MPI Fe detection threshold on the order of picograms. Recent publications report an agent specific intracellular loading of 2-9 pg Fe/cell and 13-50 pg Fe/cell for NSCs and MSCs, respectively (Bulte 2015, Zheng 2016), which suggests MPI has a sensitive cell detection threshold. This initial work indicates the spatial resolution, sensitivity, and linear signal quantification of

the MOMENTUM MPI platform could make it a potent tool for stem cell research.

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W-2143

MULTIWELL OPTOGENETICS FOR ENHANCED CONTROL OF HUMAN IPSC-DERIVED CELLS

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Optogenetic techniques enable precise optical manipulation of cells via integrated light-activated channels (opsins). Expression of light-sensitive proteins allows the user to activate or suppress activity in targeted cell types or selected biomolecular pathways. Pairing of optical stimulation with simultaneous microelectrode array (MEA) recordings provides a powerful tool for monitoring and manipulating cultured cell activity in vitro, offering insight into neuronal network interactions, stem cell characterization, toxicology screening, and drug safety and development. Here we explore various applications of Lumos, a commercial multiwell optical stimulation system, when paired with the Maestro, a system for high throughput MEA recording. In one case-study, human iPSC-derived cardiomyocytes were optically paced and challenged with test compounds in a Maestro MEA assay. Light-mediated pacing of hiPSC-cardiomyocyte activity was found to reduce well-to-well variability and allowed rate-dependent effects of test compounds to be explored. These findings demonstrate how scaling in vitro optical stimulation and MEA-based recording to high well counts enables enhanced, high-throughput studies.

W-2145

AN INDUCIBLE CRISPR-ON SYSTEM FOR CONTROLLABLE GENE ACTIVATION IN HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) are an important system to study early human development, model human diseases, and develop cell replacement therapies. However, genetic manipulation of hPSCs is challenging and a method to simultaneously activate multiple genomic sites in a controllable manner is sorely needed. Here, we constructed a CRISPR-ON system to efficiently upregulate endogenous genes in hPSCs. A doxycycline (Dox) inducible dCas9-VP64-p65-Rta (dCas9-VPR) transcription activator and a reverse Tet transactivator (rtTA) expression cassette were knocked into the two alleles of the AAVS1 locus to generate an iVPR hESC line. We showed that the dCas9-VPR level could be precisely and reversibly controlled by the addition and withdrawal of Dox. Upon transfection of multiplexed gRNA plasmid targeting the NANOG promoter and Dox induction, we were able to control NANOG gene expression from its endogenous locus. Interestingly, an elevated NANOG level promoted naïve pluripotent gene expression, enhanced cell survival and clonogenicity, and enabled hESCs to integrate with the inner cell mass (ICM) of mouse blastocysts in vitro. Thus, iVPR cells provide a convenient platform for gene function studies as well as high-throughput screens in hPSCs.

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W-2147

TARGETED OPTIMIZATION OF CHEMICALLY-DEFINED MEDIUM FOR MSC GROWTH IN BIOREACTORS

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The long-term outlook for stem cell therapy predicts an increased need for high quality animal origin-free materials compatible with limited downstream processing steps. Large scale manufacturing of human mesenchymal stromal/stem cells (hMSCs) necessitates movement toward technologies such as stirred tank bioreactors that support scalable cell culture processes. Serum-free media often fail to support robust attachment and expansion of adherent MSCs grown on microcarriers in bioreactors. The lack of small scale high throughput (HTP) models for microcarrier culture make media optimization challenging which can inhibit successful transition of planar processes into bioreactors. In this presentation we describe an approach to media optimization that uses gene expression fingerprinting to better predict media performance in microcarrier based systems. We will highlight the use of genome-wide expression analysis to identify a target transcriptome associated with successful expansion of bone marrow derived MSCs on microcarriers. In addition, we will summarize results from a high-throughput formulation DOE and multivariate analysis used to identify candidate media formulations with the target transcriptome. Finally, the utility of this approach will be demonstrated via performance of top candidate formulations identified

in planar culture that are transitioned to suspension culture in bioreactors. High quality reagents are key enabling technologies for success in commercializing cell therapies.

W-2149

CLASSIFICATION OF MULTIPOTENTIAL STROMAL CELLS USING MASSIVELY PARALLEL MONTE CARLO LINEAR DISCRIMINANT ANALYSIS OF SINGLE CELL MRNASEQ DATA

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Sufficient characterization of stem cell derived products to allow selection of in-process and release tests that predict in vivo performance reliably has been a formidable challenge. Methods in common use, including population-average molecular assays (e.g., qPCR, microarray) or flow cytometric evaluation of selected antigens, have not yielded a definitive solution to this problem.To assess whether more detailed single cell analyses might provide greater discriminating power than methods currently in use, we cultured bone marrow stromal cells (also called mesenchymal stem cells or Multipotential stromal cells (MSCs)) for varying periods at 3% or 20% oxygen. Under these conditions, the cells all display surface antigens (CD29+, CD44+, CD105+, CD166+, CD45-, CD14-, CD34-) considered characteristic of MSCs. We then analyzed them by single cell RNAseq. To overcome limitations in floating point precision and RAM size encountered with conventional computing platforms confronted with the very large data sets generated, we devised a novel computational workflow incorporating Monte Carlo sampling and massive parallelization. This allowed us to apply Fisher's Linear Discriminant Analysis (LDA), which is computationally intensive, to the data sets we generated, in which hundreds of cells were assayed for thousands of transcripts. We found that this approach was able to identify sets of transcripts comprising classifiers in the high-dimensional LDA space that distinguished between each group of cells tested unambiguously, thus affording level of discriminating power between closely similar cell populations that has not been achieved previously. We believe these findings offer the possibility a generally applicable approach to evaluating effects of cell therapy manufacturing process variables and to identification of potential molecular attributes indicative of product quality of higher discriminating power than methods currently in use.

W-2151

SENSITIVITY OF HUMAN PLURIPOTENT STEM CELLS TO INSULIN PRECIPITATION INDUCED BY PERISTALTIC PUMP-BASED CULTURE MEDIUM CIRCULATION

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Human pluripotent stem cells (hPSCs) have the potential to revolutionize biomedical sciences but their standardised mass production, indispensable for clinical use, is still a challenge. In the perspective of advanced hPSC bioprocessing, "matrix-free cell only aggregate" suspension culture within stirred-tank bioreactors represents a promising strategy. However, aggregate size heterogeneity and linear - rather than exponential - growth kinetics which may result from the hydrodynamic conditions in impeller stirred bioreactors trigger investigations into alternative technologies. Recently, an impeller-free bioreactor for dynamic suspension culture was proposed. In this system, dynamic suspension is achieved by specific hydrodynamic conditions established by continuous peristaltic pump-based medium circulation. In order to test the applicability of this platform for advanced suspension culture of hPSCs, this study investigated how specific hPSC expansion media (i.e. E8, TeSR™-E8™, mTeSR™1 and StemMACS™ iPS-Brew XF) comply with continuous peristaltic pump-based circulation. The study revealed an unexpected sensitivity of specific media components to the applied circulation mode. In particular, in low protein media E8 and TeSR™-E8™ peristaltic pumping induced physical instability of the growth hormone insulin, which precipitated into insoluble particles. The drastic reduction of insulin in the medium induced a severe viability loss in hPSC aggregates cultured in suspension, serving as a relevant cell assay. In contrast to insulin depletion, individual withdrawal of other medium

proteins such as bFGF, TGFß1 or transferrin, essential for maintaining hPSCs pluripotency, showed minor short term effects on cell viability and the integrity of hPSC aggregates. Supplementation of the surfactant glycerol or the use of the insulin analogue Aspart did not overcome the issue of insulin precipitation. In contrast, the presence of bovine or human serum albumin (BSA or HSA, respectively) stabilized insulin rescuing its content, possibly by molecular chaperone-like activity, ultimately supporting hPSC maintenance. Given the necessity to progress towards GMP-compliant media compositions and automated hPSC processing technologies, this study has substantial impact for hPSC manufacturing.

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W-2153

MITOSORT: A NEW USER-FRIENDLY MITOCHONDRIAL DNA SEQUENCING ANALYSIS TOOL FOR QUALITY CONTROL OF HIPSC CLONES

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Quality control of human induced pluripotent stem cells (hiPSCs) is crucial for the success of this technology. Genetic stability of hiPSCs is critical to avoid misinterpreting data in basic research, and to avoid unexpected complications in regenerative medicine. hiPSCs can harbor mutations in nuclear DNA, but also in mitochondrial DNA (mtDNA). In fact, the mutation rate in mtDNA is at least 10 to 20 fold higher than nuclear DNA. The genes encoded in mtDNA have a fundamental role in energy production in the mitochondria. The coexistence of multiple variants of mtDNA in a cell is referred to as heteroplasmy, and even healthy somatic mammalian cells harbor mtDNA variants at very low levels. Recently, our group showed how these mutations can be revealed through nuclear reprogramming producing hiPSC clones with damaging mutations that may lead to respiratory defect in differentiated cells. Moreover, mutations in mtDNA can be accumulated in somatic tissues during aging, and the amount of mtDNA mutations is higher in hiPSCs-derived from old individuals. These findings highlight the importance of deep characterization of mtDNA as a quality control of hiPSCs. Here, we present an open-source software package to process and analyze Next Generation Sequencing (NGS) mtDNA data with the goal to screen and identify potentially damaged hiPSC clones and prevent unintended consequences. Currently researchers use multiple tools and annotation sources to analyze mtDNA samples which are time consuming. As the throughput of mtDNA samples increases there is a greater need for a comprehensive software tool that quickly performs the analysis on a large number of samples. Our tool allows users to start with raw NGS data in either FASTQ or BAM format from PCR-targeted mtDNA sequencing, whole exome sequencing, or whole genome sequencing. Results are presented in a user-friendly report with hiPSC quality scores that allow researchers to easily assess the quality of their hiPSCs. To date we have analyzed the mtDNA sequence of 214 hiPSC clones derived from a cohort of 32 individuals (17 healthy donors, 9 patients with congenital heart disease and 6 patients with mitochondrial disease). MitoSort tool is able to quickly identify damaged hiPSC clones, even derived from healthy donors, preventing their use in further applications.

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W-2155

LIVE CELL FLUORESCENCE LIFETIME IMAGING MICROSCOPY (FLIM) OF INTESTINAL ORGANOID PROLIFERATION

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Intestinal organoids display villi-crypt organization, presence of heterogeneous epithelial cell monolayer and lumen. The complex composition of organoids includes stem and Paneth cell niches, amplification zones and differentiated enteroendocrine, enterocyte and goblet cells. While organoids represent advanced in vitro model to study development and therapy of intestine there is a lack of tools allowing their analysis in live state with single cell resolution. The organoid heterogeneity caused by nutrient, metabolite and O2 availability (hypoxia) also remains poorly studied. Here, we used fluorescence lifetime imaging microscopy (FLIM) to develop experimental approaches for organoid analysis in live culture. With the help of environment-sensitive fluo- and phoshorescent probes, FLIM allows quantitative measurements of various physiological parameters such as O2, pH or T. We found that incorporation of 5-Bromo-2′-deoxyuridine (BrdU) in cell nuclei results in prominent quenching of fluorescent dye Hoechst 33342, which can be detected by FLIM and used for detection of cells in S phase of cell cycle. Compared to antibody-aided detection, FLIM approach

is more versatile, allowing analysis of live cells in both 2D (adherent) and thick 3D tissue models, including tumor spheroids and mouse intestinal organoids. We observed strong red autofluorescence of lumen in intestinal organoids, which can interfere with certain fluorescent probes. However, we found that S phase cells, present in amplification-transition zone can be efficiently detected with FLIM. We studied whether the anti-diabetic drug metformin affects cell proliferation in intestinal organoid model. Using normalization per number of crypts, we found no significant effect of metformin on proliferation of cells in organoids. To illustrate the versatility of live cell FLIM, we combined labeling of proliferating cells with analysis of organoid oxygenation. Using staining with phosphorescent O2 probe and FLIM we found highly variable oxygenation in resting organoids, ranging from 27 to 88 uM. In conclusion, our data points that intestinal organoids differ by size, shape, number of crypts, cell proliferation and oxygenation. Our results emphasize the importance of live fluorescence imaging analysis in studies of organoids.

Funding Source: Supported by Science Foundation Ireland (SFI) grant 13/SIRG/2144.

W-2157

TP53 INHIBITION ENHANCES CRISPR/CAS9 ENGINEERING IN HUMAN PLURIPOTENT STEM CELLS BY BLOCKING DSB-INDUCED TOXICITY

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CRISPR/Cas9 has revolutionized our ability to make targeted genomic changes and to conduct genome-wide screens in human cells. While some cell types are easily modified with Cas9, human pluripotent stem cells (hPSCs) poorly tolerate Cas9 and are difficult to engineer. We developed a method for efficiently engineering hPSCs using a drug-inducible Cas9. We found that double strand breaks (DSBs) induced by Cas9 are toxic, kill the majority of hPSCs and demonstrated this response is tp53-dependent. In addition, transient inhibition of TP53 dramatically improved the efficiency of precise transgene knock-in by over fifteenfold. These results are directly relevant to the use of CRISPR/Cas9 in high-throughput genome engineering. They also suggest that groups using CRISPR/Cas9 for therapeutic applications should monitor tp53 toxicity which may cause cell loss or tissue damage, and the inadvertent selection of tp53 mutations upon engineering which poses a risk for patients.

Funding Source: Novartis Institutes for Biomedical Research

W-2159

QUANTIFYING THE CENTRAL DOGMA OF MOLECULAR BIOLOGY WITH SINGLE CELL RESOLUTION

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Despite the role of RNA and protein synthesis in governing cellular function, there remains little information on how these activities are controlled during heterogeneous processes such as differentiation. This knowledge gap stems largely from inadequate methods to quantify these processes in a high-throughput, single-cell manner. To address this problem, we developed a simple, 'one-step' method to non-specifically label and quantify nascent DNA, RNA and protein molecules simultaneously. With this, we can now measure relative amounts of newly synthesized DNA, RNA and protein in single cells by mass-cytometry (CyTOF). This is accomplished by pulsing live cells with an optimized combination of 5-iodo-2′-deoxyuridine, 5'-bromouridine, and puromycin prior to collection and fixation. Each small molecule is metabolically incorporated in DNA, RNA, or protein, respectively, and then detected using epitope-specific monoclonal antibodies or incorporated elemental label. When combined with single cell mass cytometry, DNA, RNA and protein synthesis is quantified in single cells simultaneously with 40+ parameters (i.e. phenotypic, intracellular, phospho-, etc.), and can be performed on adherent and non-adherent cells, ex vivo isolates, and should be extendible to applications in vivo. Illustrated here, we investigate the synchronization of DNA, RNA and protein synthesis in the context of cell cycle and across lymphopoiesis in a healthy human immune system. First, this method was used to highlight the progressive restriction of DNA, RNA, and finally protein synthesis as asynchronously growing HeLa cells transition from S phase through G2 and into mitosis. Second, this method was used to label DNA, RNA, and protein synthesis in healthy human bone marrow cells. Simultaneous quantification 43 phenotypic parameters were used to cluster cells across B-cell maturation. Our initial results show dynamic synthesis of RNA and protein, irrespective of proliferative status, in select progenitor and differentiated B-cell subsets. Our simple, one-step protocol for quantifying DNA, RNA and protein synthesis simultaneously with phenotypic and regulatory features in single cells can now reveal systems-level roles these fundamental cellular process play in cellular homeostasis, differentiation, and dysfunction.

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W-2161

CELL CLUSTERS COMPOSED OF INDUCED PLURIPOTENT STEM CELLS ANALYZED BY LARGE PARTICLE FLOW CYTOMETRY PARALLELS THE CHARACTERIZATION OF THE COMPONENT SINGLE CELLS

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Stem cell clusters, embryoid bodies, organoids and other types of 3D cultures have been enormously important biological systems for the discovery of signals responsible for normal development and differentiation. There is an increased interest in studying how cells grow, divide and differentiate in a more natural context provided by these 3D cell culture models. Many cell types will naturally form cell clusters when given the opportunity. This research approach allows for cell-cell interactions to occur and provides biological insights otherwise missed when studying flat sheets of cells growing on plastic surfaces or as cells grow in isolation. Furthermore, there are many methods that enable the introduction of genes for fluorescent proteins expressed from promoters of interest. Researchers can use these to identify when a cell or group of cells has transitioned to a different state. Using this approach, cell clusters can be analyzed for these types of changes and transitions, and clusters of different types isolated for further studies. One type of analysis of these cell clusters involves determining the contribution of each of the different types of cells to the clusters. The level of fluorescence in a cell cluster can be used for this determination and is a result of the number of positive cells and the intensity of light emitted from the fluorescent marker on/in those cells. We compared the analysis of the fluorescence level of an entire cell cluster with the fluorescence measurement of the individual cells of that particular cell cluster. The BioSorter® was used to measure and dispense an individual cell cluster into the well of a multiwell plate. Then, cells of the cluster were separated from each other and analysed by conventional single cell flow cytometry. We compared the fluorescence mean intensity of the cluster as measured on the BioSorter with the total number and mean channel of fluorescent cells within the cluster measured on a conventional single cell flow cytometer. Our data shows that the measurements from the cell clusters parallels that determined by single cell analysis.

W-2163

A STANDARDIZED AND CHARACTERIZED CLINICAL GRADE HUMAN PLATELET LYSATE FOR EFFICIENT EXPANSION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS

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Human platelet lysate (hPL) is rich in growth factors (GF) and nutritive elements and represents a powerful xeno-free alternative to fetal bovine serum (FBS) notably for mesenchymal stem cell (hMSC) proliferation. However, there is a large variability in hPL sources and production protocols, resulting in discrepancies in product quality, low management of product safety and poor batch-to-batch standardization. We describe here the development and the characterization of a standardized hPL prepared from transfusional grade screened normal human donor platelet concentrates (PCs), manufactured on an industrial scale (250 donors) and following a highly qualified process (clean room, trained operators, validated aseptic filtration). PCs were frozen and thawed to lyse platelets. Cell debris were removed by centrifugation and the supernatant (hPL) was recovered. Clinical grade batches of aseptic filtered hPL were characterized. By contrast to hPL prepared from a limited number of donors, we observed a robust standardization between industrial batches of hPL in terms of GF contents (bFGF, EGF, VEGF, PDGF-AB, TGF-beta1 and IGF-1), biochemical analyses (total proteins, albumin, vitamin B12 and triglycerides) and chemical parameters (osmolality and pH). We also documented the stability over time of hPL stored at -80°C and -20°C in terms of GF contents and chemical parameters. Then we showed that clinical grade hPL enables an increase and batch-to-batch reproducible proliferation of bone marrow (BM)-hMSCs versus MSC-screened FBS (+/- bFGF). We compared the expression level of a large panel of membrane markers between hPL- and FBS-expanded hMSCs using RT-qPCR and flow cytometry analysis and observed that their variation between batches was higher in FBS conditions than in hPL conditions. We also documented an over expression of a number of membrane markers in hPL conditions (mRNA and protein levels). Finally, using quantitative methods, we observed a similar adipogenic and osteogenic differentiation potential and that immunosuppressive properties of BM-hMSCs (inhibition of T-cell proliferation) cultivated in parallel in both conditions remained identical. In conclusion, we demonstrated the feasibility to use a standardized,

efficient and clinical grade hPL for research and cell therapy applications.

Funding Source: Judith LORANT received financial support from French government (National Research Agency), Nantes Métropole and the Région Pays de la Loire.

W-2165

EFFICIENT LARGE-SCALE 2D CULTURE SYSTEM FOR HUMAN PLURIPOTENT STEM CELLS AND DIFFERENTIATED CARDIOMYOCYTES

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Cardiac regenerative therapy using human induced pluripotent stem cells (hiPSCs) is a potentially promising strategy for patients with heart disease, but the inability to eliminate residual hiPSCs and generate a massive amount of pure cardiomyocytes has been a barrier to realizing this potential. Recently, we established a novel method for purifying the bulk of hiPSC-derived cardiomyocytes by focusing on glucose, glutamine and lactate metabolism in hiPSCs and differentiated cardiomyocytes (Tohyama S, Cell Metabolism 2016). However, there are no efficient two-dimensional culture systems to obtain a large amount of pure cardiomyocytes. Here, we developed an advanced two-dimensional culture system using multilayer culture plates with active gas ventilation that yielded a large number of hiPSCs and pure cardiomyocytes. One million hiPSCs per one culture plate were cultured in ten-layered culture plates with active gas ventilation, and they stably proliferated and maintained pluripotency. As a result, 1–2 × 10E9 hiPSCs were harvested in one week. Additionally, hiPSCs were sequentially differentiated into cardiomyocytes in a 2D differentiation protocol. The efficiency of cardiac differentiation was 50–70% on average. Approximately 3–5 × 10E8 cardiomyocytes were obtained in four-layered culture plates with active gas ventilation. After metabolic purification with glucose- and glutamine-depleted and lactate-supplemented media, a large amount of pure cardiomyocytes was finally prepared. This advanced cell culture system will facilitate the clinical application of iPSC-derived cardiomyocytes.

Funding Source: Work was mainly supported by the Highway Program for Realization of Regenerative Medicine from the Japan Science and Technology Agency.

W-2167

TOOLS TO VALIDATE, COMPARE, AND OPTIMIZE CAS9-CRISPR GENOME EDITING METHODS AND REAGENTS IN HUMAN PLURIPOTENT STEM CELLS

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Precise genome editing in hiPSCs can suffer from low efficiencies even when using the often highly efficient Cas9-CRISPR approach. For some loci, efficiencies below 1% have been reported, thus requiring the analysis of hundreds of clones. Here we describe two independent reporter systems and use them to compare different genome editing approaches, reagents, and methods. The first system (plasmids developed and kindly provided by Susan Byrne/George Church) entails the replacement of the endogenous hCD90 coding exons with those of the mCd90 gene by homology-directed repair (HDR) using a conventional targeting vector lacking a drug selection cassette. Precisely targeted hiPS cells or colonies can easily be identified by mCd90/hCD90/SSEA5 triple color live immunoflurescence imaging or flow cytometry. Live imaging also reveals whether clones or lines are mixed (containing targeted and untargeted cells), allowing stem cell biologists to optimize their procedures and hone their skills. We use this system to compare the relative efficiencies of several nucleic acid delivery systems (lipofection, nucleofection) and Cas9 sources (EF1alpha promoter driven Cas9, CAG promoter driven Cas9-GFP, Cas9 modified mRNA). This system also allowed us to optimize the ratio of targeting vector, gRNA construct, and Cas9. We show that the quality of the transfected plasmid DNA preparations is a major contributing factor determining targeting efficiencies. Using FACS sorting of Cas9GFP positive cells we were able to achieve targeting efficiencies of ~80%. We also generated a second system that allowed us to similarly optimize oligo-directed repair (ODR). To this end we first generated an indel in the GFP portion of the OCT4-GFP knock-in hES cell line H1OGN (efficiency >50%) that removed the GFP chromophore encoding codons. We then desinged gRNAs to target the GFP indel of one clone and restored the GFP ORF by Cas9/CRISPR ODR using a ssDNA oligo. Targeting efficiencies were assessed by GFP fluorescence microscopy and FACS, and Sanger sequencing confirmed the presence of diagnostic SNPs in the targeted locus. We believe these tools could be valuable for hPSC CRISPR training courses, to systematically optimize genome editing protocols, and to perform chemical or genetic screens to identify modifiers of DNA repair processes in hPSCs.

W-2169

TOXICOLOGICAL RESPONSES IN CULTURED HUMAN IPSC-DERIVED NEURONAL NETWORKS USING HIGH-THROUGHPUT MEA SYSTEM

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The functional network of human induced pluripotent stem cell (hiPSC)-derived neurons is a potentially powerful in vitro model for evaluating drug toxicity. Epileptiform activity is one of phenomena in neuronal toxicology. To evaluate the dynamics of epileptiform activities and the effect of anti-convulsant drug in cultured hiPSC-derived neurons, we used the high-throughput multielectrode array (MEA) system, where we simultaneously record extracellular potentials for 16 channels per well across 24-well plates. We firstly confirmed the modulation of activity by typical glutamatergic and GABAergic receptor antagonists/agonists in spontaneous firings. Spontaneous activities and typical responses against synaptic related drugs were detected with high S/N ratio using high-throughput MEA system. Next, we examined chemically evoked epileptiform activity. Electrophysiological seizes were induced by pentylentetrazole (PTZ), 4-Aminopyridine (4-AP), and kainic acid (KA), the most widely used chemical convulsant in animal models to screen for new anti-epilepsy drugs. We also examined the anti-convulsant effects of common clinical anti-epilepsy drugs (AEDs), phenytoin. PTZ, 4-AP and KA induced an increase in synchronized burst firings (SBFs) in a concentration-dependent manner. Phenytoin suppressed induced epileptiform activity. However, the patterns of epileptiform activities and phenytoin effects were different with respect to each epilepsy drugs. From these results, we suggest that the electrophysiological assay in cultured human iPSC-derived neuron using high-throughput MEA system is a useful to investigate the neuronal toxicity in drug screening and pharmacological effects of human neurological disease.

W-2171

HIGH-THROUGHPUT EXTRACELLULAR MATRIX MICROENVIRONMENTS FOR PROBING ENDOTHELIAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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The basement membrane extracellular matrix (ECM) plays an important role in modulating endothelial cell function and phenotype. In order to harness the interaction effects between endothelial cells (ECs) and their surrounding ECM for vascular tissue engineering, we systematically examined the role of combinatorial ECMs on endothelial differentiation using an arrayed microscale platform. ECM microarrays were developed by covalent conjugation of basement membrane proteins (gelatin (G), fibronectin (F), laminin (L), heparan sulfate proteoglycan (H), collagen IV (C), and matrigel (M)) and all the multi-component combinations thereof, onto glass slides. Endothelial differentiation was induced on the microarray using human induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs). After 5 days of differentiation on these unique combinatorial ECM environments, we observed significantly higher CD31 expression when the cells were differentiated on collagen IV + gelatin + heparan sulfate (CGH) combinatorial ECMs, compared to other combinations across three human pluripotent stem cell lines ($N=13$; $p < 0.05$). This enhancement in endothelial differentiation on an arrayed microsystem was confirmed under conventional cell culture platforms in which a marked relative increase in CD31 expression was observed in CGH modified-dishes (201.1±11.18%, $N=5$; $p < 0.01$), compared to collagen IV-modified dishes (100±0%, $N=5$). To elucidate the role of cell-ECM interactions on endothelial differentiation, the temporal expression pattern of endothelial phenotypic genes was matched by the similar expression pattern of integrin subunits β3 (51.69±13.28 fold, $N=3$; $p < 0.01$), relative to undifferentiated ESCs. To demonstrate the functional importance of integrin β3 in promoting endothelial differentiation, the addition of neutralization antibody abrogated the enhancement of endothelial differentiation on CGH modified-dishes. Together, these findings demonstrate that combinatorial ECMs CGH promote higher levels of endothelial differentiation, compared to many single-factor ECMs, in part through integrin β3-mediated pathways. This work highlights the importance of combinatorial cell-ECM interactions in modulating stem cell fate.

W-2173

AN AUTOMATED WALK-AWAY SYSTEM TO PERFORM DIFFERENTIATION OF 3D MESENCHYMAL STEM CELL SPHEROIDS

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Human mesenchymal stem cells (hMSCs) are a type of multipotent stem cell found in multiple areas of the body including bone marrow, skeletal muscle, dermis, and blood. The cells are highly sought after due to their ease of isolation, ability to differentiate and mature into multiple lineages, and critical role in tissue engineering

and adult tissue repair. One area of particular interest is using chondrocyte derived hMSCs for adult cartilage repair. Initial experimentation with hMSCs involved two-dimensional (2D) culture of cells in a monolayer. However, culturing the cells in this manner resulted in a loss of replicative ability, and differentiation capability over time. A number of techniques to culture hMSCs in a three-dimensional (3D) format were then incorporated, such as pellet and micromass culture. These methods better exemplified the differentiation process, but requiring large cell numbers, involved difficult processing steps, and were expensive. Newer 3D cell culture technologies, where spheroids of smaller cell number in high density microplates can overcome earlier limitations and still provide the necessary stem cell differentiation environment. Complete differentiation from multipotent hMSCs to final target lineages, such as chondrocytes, typically takes 14-28 days. Performing media exchanges every 2-3 days with unattached cells can be tedious and risks spheroid removal. Adding automation allows for performance of other tasks and increases repeatability. When combined with 3D magnetic bioprinting, where magnetic nanoparticles attach to spheroidal cells, spheroid loss is eliminated by placing test plates onto magnets during media exchanges. Here we demonstrate a solution to perform automated chondrocyte differentiation from 3D hMSC spheroids. A combination washer/dispenser with magnetic plate adapter was used for media exchanges, while an automated incubator maintained proper microplate environmental conditions in between exchanges. Label-free cellular imaging following media exchanges confirmed maintenance of spheroids during processing. The entire system was contained within a laminar flow hood. Immunofluorescence carried out following differentiation confirmed the ability of the system to be used for critical stem cell differentiation.

POSTER SESSION I-EVEN 19:30 – 20:30

PLACENTA AND UMBILICAL CORD DERIVED CELLS

W-1002

NLRP3 AS A NOVEL REGULATOR OF HUMAN MESENCHYMAL STEM CELL FUNCTION

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Inflammasome is a multimeric protein complex which senses inflammatory stimuli. Mesenchymal stem cells (MSCs) are promising tool for regenerative medicine and immune-related diseases. Previous reports demonstrate that MSC function can be altered by stimuli derived from innate and adaptive immunity. In the present study, we investigated the expression and functional regulation of inflammasomes in human umbilical cord blood-derived MSCs (hUCB-MSCs). The hUCB-MSCs expressed the components of inflammasomes. Among several types of inflammasomes, NLRP3 activation did not alter the characteristics of hUCB-MSCs nor induced the pyroptosis of MSCs. Surprisingly, NLRP3 activation promoted the proliferation and osteogenic differentiation of hUCB-MSCs. Moreover, the immunomodulatory effects of MSCs on T cell proliferation, dendritic cell (DC) and regulatory T cell were changed in response to NLRP3 activation. In addition, the expression levels of immunomodulatory factors were elevated in hUCB-MSC after NLRP3 stimulation. In conclusion, for the first time, our data suggest that NLRP3, one of the inflammasome family, is expressed in hUCB-MSCs and its activation can regulate the functions of hUCB-MSCs along with the up-regulation of multiple soluble factors.

PLACENTA AND UMBILICAL CORD DERIVED CELLS

W-1004

GMP ISOLATION AND IMMUNOMODULATORY CHARACTERIZATION ON HUMAN AMNION EPITHELIAL CELL FOR CLINICAL TRANSPLANTS

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Placenta is a non-controversial and readily available source of stem cells for regenerative medicine. We previously reported that human amnion epithelial cells (hAEC) from term placenta are not tumorigenic, have immunomodulatory and anti-inflammatory properties and once transplanted differentiate into functional hepatocyte-like cells. In preclinical studies with immune-competent mice, hAEC engrafted and survived without administration of immunosuppressive drugs, resulting in correction of metabolic liver diseases (iMSUD and PKU) or the reversal of acute liver failure. Amnion characteristically lacks HLA class 2 expression and expresses both class 1a and non-canonical class 1b. Expression of the class 1b proteins by placenta supports maternal immune-toleration of the fetus. Recently, purinergic mediators, hydrolyzed by plasma membrane nucleotidases, have also been shown to regulate immune cell response. We quantified the level of expression of both HLA molecules and ecto-enzymes in hAEC preparations. hAEC from 20 full term placentae have been isolated in accordance with current Good Manufacturing Practice (cGMP). Flow cytometric evaluation routinely validates hAEC identity. Ectonucleotidases CD39 and CD73, and HLA molecules were measured on all preparations, after isolation and pre-transplant. Immunogenicity of the hAEC was determined on purified immune effector cells (T-, B- and NK-cells). hAEC preparations were positive for epithelial markers (CD49f and EpCAM), and negative for hematopoietic and stromal markers. We measured high level of expression for ectonucleotidases (CD39 and CD73) in all hAEC preparations. The results showed the constitutive presence of both membrane-bound and soluble HLA-G isoforms. Ongoing analyses are focused on confirmation in HLA class 1b expression after long-term engraftment in immunocompetent animals. High level expression of ecto-enzymatic axis and non-canonical HLA molecules likely play a key role in immunological tolerance and long-term acceptance of the human xeno-cell graft in immunocompetent mice. Based on their safety and the successful preclinical studies, approval was granted to begin isolation and banking of hAEC under cGMP procedures at Karolinska Institutet, and to perform hAEC transplants on up to 10 patients with liver disease.

ADIPOSE, MUSCULOSKELETAL, AND CONNECTIVE TISSUE

W-1006

ADULT STEM AND PROGENITOR CELL HETEROGENEITY IN HUMAN BONE AND ADIPOSE TISSUES

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Advancing the understanding of the heterogeneous population of native stem and progenitor cells (Connective Tissue Progenitors/CTPs) in different tissues and batches of culture expanded cells is key to define the ideal cell source to use in cell therapy. The aim of this study was to compare sources and characterize the biological features of human CTPs derived from bone and adipose tissues in primary culture and after culture expansion to passage 2 (P2). Cancellous bone and subcutaneous adipose tissues were collected from 8 patients undergoing hip arthroplasty in an IRB approved protocol. Three cell fractions were isolated per patient: 1) MS, bone-derived marrow space; 2) TS, bone-derived trabecular surface; and 3) AT, adipose tissue-derived cells. We assessed colony formation using ColonyzeTM software to determine CTP prevalence (PCTP), colony metrics, and cell counts; and phenotypic characteristics using flow cytometry to determine percentage of cells expressing the classical MSC surface markers: CD73, CD90, CD105, as well as CD146, E-cadherin, Ep-CAM, hyaluronan; and pluripotency markers: Oct3/4, Sox-2, Nanog, SSEA-4, SSEA-3, Cripto-1. Mean PCTP and cell density in TS fraction were significantly higher ($p = 0.0003$ & $p = 0.0002$, respectively) than in MS. Mean total adherent cells/10^6 cells plated was significantly different between tissue sources, with AT > MS ($p = 0.01$) and TS > MS ($p < 0.0008$). P2 cells showed similar doubling time. All P2 cell sources expressed classical MSC markers >95%. However, large variations were observed between patients and tissue sources in all

other markers. Greater emphasis needs to be placed on establishing critical quality attributes based on other markers that will be predictive of future biological behavior and therapeutic potency of stem/progenitor cells. Differences between tissue sources and the heterogeneity in stem/progenitor cell populations derived from various tissues is an untapped opportunity for improving the performance of culture expanded cells. The prevalence and biological potential of CTPs are different from one patient and one tissue to another. The lack of variation in classical MSC markers limits their value as metrics of quality. Other markers are much more likely to discriminate differences between cell populations in biological performance.

W-1008

ISOLATION AND DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS FROM FAT TISSUE BY MEMBRANE FILTRATION METHOD

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Human adult stem cells, such as human adipose-derived stem cells (hADSCs), are an attractive source of stem cells in regenerative medicine. However, the isolated hADSCs possess different purity levels and divergent properties depending on the purification methods used. We developed a hybrid-membrane migration method that purifies hADSCs from a fat tissue solution with extremely high purity and pluripotency. A primary fat-tissue solution (SVF) was permeated through the porous polymeric membranes with a pore size from 8 to 25 um, and the membranes were incubated in cell culture medium for 15-18 days. The hADSCs that migrated from the membranes contained an extremely high percentage (e.g., >98%) of cells positive for mesenchymal stem cell markers and showed one order of magnitude higher expression of some pluripotency genes (Oct4, Sox2, Klf4 and Nanog) compared with cells isolated using the conventional culture method. We found that we could successively purify hADSCs by the hybrid-membrane filtration method using (a) PLGA/silk membranes with a pore size (r)=18.2-24.4 um, (b) nitrocellulose (NC-8, r=8 um), (c) nylon mesh filter (NY-11, r=11 um) and (d) polyurethane (PU-11, r=11 um). The porous membranes having variety of synthetic materials could be used in this method. However, porous membranes made of extracellular matrices (ECMs) and membranes coated with ECMs was less effective to use in the purification of hADSCs because of hADSCs adhesion on ECMs on the membranes. Another important characteristics of the membranes is the pore size. Almost no permeation of the primary fat-tissue solution (SVF) was observed through the membranes having less than 8 um. When the nylon mesh filter having r=20 um was used, we could collect hADSCs by the membrane filtration method. Furthermore, the yield of hADSCs became less than 10% using the nylon mesh filter having r=40 um compared to that purified using NY-11 from SVF solution. Therefore, the optimal pore size of the membranes used in the hybrid-membrane filtration method was determined in the range of 8-25 um. hADSCs purified by the hybrid membrane migration method showed much better osteogenic differentiation ability than hADSCs isolated from the conventional culture method.

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W-1010

PEPTIDE YY RECAPITULATES THE PROLIFERATIVE EFFECTS OF BFGF IN HUMAN MUSCLE PROGENITOR CELLS IN LATE BUT NOT EARLY PROLIFERATION

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Satellite cells (SCs), skeletal muscle specific stem cells, are integral to regeneration after injury or stress. Microenvironment and systemic factors regulate SC function and thus regeneration. Basic fibroblast growth factor (bFGF) and high levels of serum facilitate proliferation in cultured SCs (muscle progenitor cells, MPCs). Peptide YY (PYY), which we have detected in MPCs, may also play an important role. In proliferative MPCs, PYY levels decrease after 168 h of proliferation. After 72 h in normal growth medium, replacing bFGF with exogenous PYY for 96 h promotes proliferation. Exogenous PYY stimulates proliferation at the same time-point endogenous PYY levels decline. Therefore, our objective was to determine if the pro-proliferative effect of PYY in the absence of bFGF is temporal in nature. Human SCs were obtained from the vastus lateralis of young (22-40 y) females (n = 5) and sorted by FACS using SC specific markers. MPCs were cultured in a modified growth medium (GM) of Ham's F12, 20% fetal bovine serum, and 1% antibiotics supplemented with or without 5 ng/mL bFGF and 0.5 nM PYY creating four conditions: 1) GM (GM-only); 2) GM + bFGF (GM+bFGF); 3) GM + PYY (GM+PYY); and 4) GM + bFGF and PYY (GM+bFGF+PYY). Medium was replaced every 24 h for 10 d. Every 24 h an imaging cytometer was used to measure confluence and count live/dead cells after co-staining with Hoechst 33342 (nuclei) and Propidium Iodide (dead cells). Confluence was not

different among the conditions tested (P>0.05). After 168 h of growth, GM+bFGF and GM+bFGF+PYY had more nuclei (approximately double) than GM-only (P < 0.05) and GM+PYY (P < 0.05). All medium resulted in < 10% cell death. Cell death was lower in the GM+bFGF medium compared to GM-only (P < 0.05), but cell death in GM+bFGF was not different from GM+PYY (P>0.05). Exogenous PYY did not rescue proliferation when MPCs were seeded in medium without bFGF. Despite a greater number of nuclei with bFGF containing medium, the confluence increased similarly for all conditions, suggesting increased cell size in cultures lacking bFGF. While PYY and bFGF may both maintain MPC proliferation, exogenous PYY alone cannot initiate proliferation. Ongoing studies will elucidate the temporal relationship between endogenous and exogenous PYY on MPC proliferation and mechanisms driving this relationship.

Funding Source: Canadian Institute of Health Research Doctoral Foreign Study Award

W-1012

THE 2-OXOGLUTARATE ANALOGUE DMOG COMPARED TO FE2+ DEPLETION ENHANCES CHONDROGENESIS OF HUMAN BONE MARROW MESENCHYMAL STROMAL CELLS VIA HIF-1A

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The chondrogenic differentiation of bone marrow mesenchymal stromal cells (BM-MSC) is regulated by hypoxia, the effects of which are mediated by the hypoxia inducible factor (HIF) transcription complex. HIF at normoxia is downregulated by the hydroxylases prolyl hydroxylase 2 (PHD2) and factor inhibiting HIF (FIH), which both utilise Fe2+ and 2-oxoglutarate (2-OG) to target the HIF-1α subunit. Stabilisation of HIF-1α using PHD2 inhibitors may therefore be an effective means to control HIF activity and chondrocyte differentiation for cartilage tissue engineering. We compared the effects of hydroxylase inhibitors on human BM-MSC from healthy paediatric donors, cultured in Transforming Growth Factor β3-containing chondrogenic media (TGFβ3-CDM), examining HIF-1α function and chondrogenesis by qRT-PCR and immunohistochemistry. The 2-OG analogue, dimethyloxalylglycine (DMOG), and compounds which reduce Fe2+ availability (cobalt chloride and deferoxamine) mediated similar levels of HIF-1α nuclear localisation. Only DMOG however, increased mRNA expression of chondrocyte markers, SOX9 (p=0.0240), COL2A1 (p=0.0278) and enzymes required for collagen matrix assembly, LOX (p=0.0027), P4HA1 (p=0.0039) whilst reducing mRNA encoding COL10A1, a hypertrophic marker (p=0.0037). These effects were abolished by the HIF-1α inhibitor, acriflavine, indicating that reduction in 2-OG availability, but not Fe2+, increases the function of HIF-1α in the HIF complex, without altering HIF-1α protein levels. FIH acts to inhibit binding of co-factors to HIF-1α, as opposed to PHD2 which reduces HIF-1α protein stability. Therefore our observations suggest an effect on FIH and not PHD2 in governing expression of HIF targets in BM-MSC. Despite DMOG's role in upregulating the expression of chondrogenic mRNA, immunohistochemical staining showed decreases in cartilage-like matrix formation compared to that induced by TGFβ3-CDM only. However, treating BM-MSC with DMOG for the last 7 days of chondrogenic differentiation resulted in mRNA expression similar to that in response to constant treatment, but without reduced collagen type II protein formation. Taken together, these data suggest the use of 2-OG analogues to regulate HIF activity in BM-MSC, and direct cartilage regeneration for repair of acute chondral defects.

Funding Source: Orthopaedic Research UK

W-1014

A POSITIVE FEEDBACK MEDIATED BY TWO RNA BINDING PROTEINS REGULATES MYOGENESIS

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Myogenesis is a complex process orchestrated by many factors to precisely regulate the proliferation and differentiation of muscle stem cells. The transcriptional regulatory network knitted by key myogenesis related transcription factors such as MyoD and myogenin has been well documented. The post-transcription regulation steps have emerged to play critical roles in myogenesis regulation. MyomiRs (miR1, miR133, and miR206) and other miRs have been shown to be among the key regulators of myogenesis. However, the role of RNA binding protetins coupled miR processing in myogenesis has not been well documented. We found that RNA binding protein HuR and Msi2 work coordinately to regulate the processing of microRNAs. HuR forms the basic scaffold to recruit another RNA binding protein Msi2. The amount of Msi2 serves as a trigger for the processing of pri-miR7. Sufficient amount of Msi2 will prevent the processing of pri-miR7 and facilitate muscle stem cell differentiation. To initiate this

process, more HuR protein was exported to cytoplasm. The cytoplasmic HuR can bind the 3' UTR of Msi2 and facilitate its translation. More Msi2 protein was produced and imported to the nuclei. Therefore, more Msi2 was recruited to the Msi2-HuR complex to prevent miR7 processing and further enhance differentiation of muscle stem cells. The positive feedback circuit mediated by HuR and Msi2 facilitate the efficient differentiation of muscle stem cells.

W-1016

APOLIPOPROTEIN A-I PINPOINTED BY A HIGH THROUGHPUT SCREEN PREVENTS OSTEOPOROSIS IN TRANSGENIC MICE AND TREAT OSTEOPOROSIS BY THE APPLICATION OF I-BET151

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By a high throughput screen with 12380 cDNA, we discovered that ApoA-I could significantly promote osteogenic differentiation of human primary mesenchymal stem cells in vitro. ApoA-1 transgenic mice increase the CFU-ALP, while knockout mice decrease it. Elevated ApoA-I expression efficiently prevented osteoporosis in ovariectomized mice that mimic postmenopausal osteoporosis in human. Interestingly, the treatment of I-BET151, an ApoA-1 inducer, significantly treat osteoporosis. The bone density of the mice is equivalent to the wild-type mice that performed the sham surgery control. ApoA-I promoted osteogenesis through concurrently increasing osteoblast numbers and inhibited osteoclastogenesis through decreasing osteoclast numbers. Of note, ApoA-I significantly increased the expression amounts of CXCL 6 and 8 during osteogenesis that is mediated through STAT3. Both CXCL6 and CXCL8 are essential for ApoA-I-mediated osteogenesis. However, only CXCL6 but not CXCL8, is sufficient to trigger osteogenesis in the absence of ApoA-I. Thus, although different chemokines have the same cognate receptor(s), different chemokines might still have different ability to alter MSC differentiation. Therefore, the roles of chemokines in osteogenesis remain unclear. We will perform transcriptomic analysis to measure the effects of additional chemokines on osteoporosis.

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W-1018

VASCULAR ENDOTHELIAL GROWTH FACTOR MAY IMPAIR PRO-CHONDROGENIC ACTIVITY OF PLATELET RICH PLASMA ON HUMAN ADIPOSE STEM CELLS

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Autologous cell-based repair of cartilage injury, e.g., autologous chondrocyte implantation (ACI), is limited by donor site morbidity and the need for ex vivo chondrocyte expansion. Mesenchymal stem cells (MSCs), which are more accessible and have extensive expansion and chondrogenic potential, represent an alternative cell type for cartilage repair. Platelet-rich plasma (PRP), a popular biologic-based treatment for injured/inflamed articular joint, has previously been shown to promote stem cell proliferation and tissue healing. To test the effect of PRP on MSC chondrogenesis, MSCs were isolated from infrapatellar fat pad (IFP-ASCs) of arthroplasty donors and PRP was collected from anticoagulated human whole blood and activated with CaCl2. For pellet culture, IFP-ASCs were pelleted in chondrogenic medium supplemented with different PRP concentrations (1, 5, 10, and 20%) for different durations (1-, 3-, 7-, and 21-day pulse at the beginning of culture period). For 3D culture, IFP-ASCs were resuspended in methacrylated gelatin/hyaluronic acid and photopolymerized as hydrogel constructs. Our results showed that increasing duration of PRP exposure corresponded to decreased expression of collagen type II (COL2) and aggrecan (ACN), while varying PRP treatment duration did not affect DNA content, but proportionally decreased GAG/DNA content in IFP-ASC pellet cultures. Histological examination showed that increasing PRP treatment time led to decreasing deposition of cartilage-specific extracellular matrix in IFP-ASC pellets, including proteoglycans and COL2. Similar results were observed in 3D hydrogel cultures. As vascular endothelial growth factor (VEGF), a growth factor found in PRP, has been suggested to impair chondrogenesis and cartilage repair, its involvement in PRP action was tested here by examining the effect of VEGF immuno-neutralization in IFP-ASC pellet cultures. Our results showed that at day 7, ACN gene expression decreased in the PRP group but addition of anti-VEGF antibody ablated this reduction. Taken together, our findings suggest that although PRP is reported to be beneficial for pain relief and joint function improvement, it may not enhance hyaline cartilage formation, likely due to the adverse effect of VEGF on chondrogenic differentiation.

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W-1020

MOLECULAR MECHANISMS OF CELL ADHESION AND ACTIN TENSION IN THE REGULATION OF HUMAN ADIPOSE STEM CELL ADIPOGENIC AND OSTEOGENIC DIFFERENTIATION

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Cell adhesion and cytoskeletal tension are known to guide the differentiation of mesenchymal stem cells. Cell adhesion is mediated mainly through integrins and focal adhesion proteins, such as focal adhesion kinase (FAK). FAK is interconnected with signaling pathways including extracellular signal-regulated kinase (ERK) and cytoskeleton -related Rho-Rho-associated protein kinase (ROCK) pathway and its downstream mediator myocardin related transcription factor-A (MRTF-A). However, the role and interplay of these mechanisms in the regulation of human adipose stem cell (hASC) differentiation remains unclear. The present study assesses these cell signaling mechanisms in hASC differentiation by using small molecule inhibitors against FAK, ERK and ROCK-MRTF-A pathways. Differentiation capacity of the hASCs is evaluated under basic, osteogenic and adipogenic media using RT-PCR, and analyses of alkaline phosphatase activity, matrix mineralization and lipid accumulation. Our results indicated that the inhibition of FAK, ERK and ROCK function suppressed both cell proliferation and osteogenic differentiation dose-dependently suggesting that the activity of these pathways is required for the osteogenic fate of the hASCs. ERK inhibition was found to reduce the adipogenic differentiation as well. FAK suppression decreased the cell density, while adipogenesis of the remaining cells was stimulated. Inhibition of cytoskeletal tension with ROCK inhibition resulted in increased adipogenesis, and the MRTF-A inhibition was also shown to moderately enhance the adipogenic outcome. These results suggest that the cell adhesion and actin tension modulated by FAK, ERK and Rho-ROCK-MRTF-A pathways are relevant regulators of differentiation fate of hASCs.

W-1022

METABOLOMICS AS A SENSITIVE TOOL TO ASSESS THE EFFICIENCY OF UMBILICAL CORD BLOOD MESENCHYMAL STEM CELL OSTEOGENIC DIFFERENTIATION

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Musculoskeletal regeneration with the use of umbilical cord blood mesenchymal stem cells (UCBMSCs) holds great clinical potential due to their high proliferation capacity, low immunogenicity and the possibility of autologous and off-the-shelf use. Current knowledge on the metabolic transitions during the osteogenic induction of MSCs is limited to energy production pathways and the differences between the metabolic effects of various osteoinductive agents still remain unclear. Herein, we utilize gas chromatography-mass spectrometry for the metabolomics analysis of UCBMSCs differentiated into the osteoblastic lineage under the effect of two commonly used osteoinductive agents, dexamethasone and BMP-2. Metabolism and differentiation of UCBMSCs was assessed at day 7, day 14 and day 21 after induction with 100 nM dexamethasone or 100ng/mL BMP-2 and compared to the metabolism of undifferentiated MSCs and primary osteoblasts. Culture mineralization was lower in the BMP-2 group at the end of differentiation ($P < 0.001$) and BMP-2 failed to increase alkaline phosphatase (ALP) activity at all time points throughout the differentiation process ($P < 0.01$). The superior osteoinductive efficiency of dexamethasone was subsequently reflected by metabolomics, where in contrast to BMP-2, dexamethasone profiles demonstrated high similarity to osteoblast profiles on principal component analysis and hierarchical clustering. Significance analysis of microarrays and on-network representation showed that apart from a reduction in glycolytic activity and increase in oxidative phosphorylation towards the end of the differentiation, each agent resulted to a differential pattern of non-linear metabolic shifts in glutaminolysis, tricarboxylic acid cycle amino acid pools, threonine and one-carbon cycle metabolism. Those results show discrepancies in the osteogenic efficiency of the two agents are reflected into the metabolic profiles with the use of metabolomics. Moreover, complex fluctuations in metabolic pathways during osteogenic differentiation dictate revisiting of current culture strategies to enable

customized, metabolism-tailored optimization of culture protocols.

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W-1024

HUMAN IPSC-DERIVED NOTOCHORDAL CELLS IN PORCINE IVD DEGENERATION MODEL

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A link between back pain, experienced by 80% of the adults and intervertebral disc (IVD) degeneration was established in 40% of patients. Initiation of the degeneration of the disc originates in the nucleus pulposus (NP). As an avascular tissue, the NP possesses minimal regeneration capacity. Notochordal cells (NCs) are involved in NP development and homeostasis, but disappear during maturation. The disappearance of NCs correlates with the initiation of degeneration. Human NCs are in short supply, therefore in this study we generate them from iPSCs (iNCs). Human iPSCs, reprogrammed from normal dermal fibroblasts using non-integrating plasmids, were treated with GSK3i to induce differentiation towards Primitive Streak cells (PS). Following the treatment, the iPSCs changed their morphology, PS markers were upregulated and pluripotency markers were downregulated. Then PS cells were nucleofected with Brachyury encoding plasmid, encapsulated in a hydrogel that mimics the NP environment and cultured in hypoxia (2% O2) and NP-specific media. The gene expression analysis showed the cells acquired and retained the NC phenotype for up to 8 weeks. The iPSC-derived NCs (iNCs) were co-cultured with mesenchymal stem cells (MSCs) in 1:1 ratio and MSCs' expression of the NP markers increased. To evaluate the iNC paracrine effect, MSCs encapsulated in alginate beads were grown in iNC-conditioned media (iNC-CM) and induced significantly higher expression of NP markers comparing to primary porcine NC-CM. IVD degeneration in pigs was induced by annular puncture and detected by MRI and histology. The iNCs were labeled with DiI and injected into the NP 6 weeks post annular injury. The IVDs were harvested 4 weeks after injection. The iNCs were detected and IF stains showed that their NC phenotype was consistent throughout the study. The next step will be to co-inject the iNCs with MSCs and to induce NP regeneration. We show differentiation of iPSCs to functional iNCs following the developmental pathway. No inflammation was observed in the disc, reassuring the notion that NP is immunoprivileged organ. The iNC affect MSCs in a paracrine manner, however they most probably do not differentiate to NP cells in short period of time tested. The iNCs have the potential to rejuvenate the NP, one of the most commonly degenerating tissues of the body.

CARDIAC TISSUE

W-1026

NOVEL BIOLOGICAL ROLE OF SUBSTANCE P; A BOOSTER FOR PROMOTES RIGHT ATRIUM ENDOGENOUS C-KIT+STEM/PROGENITOR CELL ACTIVATION VIA CXCR7-C-KIT EXPRESSION

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To improve the effective cardiac stem/progenitor cell (CSC) therapy for the infarcted heart, we need to challenge two factors: 1) chemical modulation using agents; 2) strategies to enhance stem cell ability and engraftment of both endogenous and exogenous sources of CSCs. We demonstrate the unknown action of neuropeptides substance P (SP) on c-Kit+CSCs. Using a Sprague-Dawley rat ischemia/reperfusion-injury (I/R) model at 1 day, we noted that endogenous c-Kit+CSCs dramatically expanded in only the right atrium (RA) of I/R with SP injection, which expresses higher levels of c-Kit, pluripotency-associated markers, and CXCr7/SDF-1α via NK1R activation, compared with I/R groups. Consistently, the SP/NK1R pathway up-regulates Akt/CXCr7 and c-Kit activation, which resulted in enhanced stem cell properties of c-Kit+CSC proliferation, migration, cardiosphere formation, and potential to differentiate into cardiomyocytes in vitro. Our findings reveal a novel insight into how SP/NK1R signaling may open possible enhancing strategies to target the basic underlying endogenous RA CSCs-mediated cardiac repair following I/R.

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W-1028

HUMAN IPS CELL-ENGINEERED HEART TISSUE TO REPRODUCE "TORSADE DE POINTES" ARRHYTHMIA IN VITRO

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"Torsade de Pointes" (TdP) is a serious arrhythmia that compromises clinical health care and drug discovery. Although numerous attempts to reproduce arrhythmias in vitro have been made with ion channel transgenic cell models or human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes, such simple 2-dimensional (2D) cell culture system was insufficient to reproduce actual occurrence of TdP. Here we show an in vitro drug-induced TdP model using hiPSC-derived cardiac tissue sheets (CTSs). With the use of temperature-responsive culture dishes (UpCell; CellSeed, Tokyo, Japan), we prepared CTSs as a 3D structure that contained a mixture of hiPSC-derived cardiomyocyte and non-myocytepopulations. We simultaneously monitored the extracellular field potential (EFP) of CTSs with a multi-electrode array and the cellular movement of contraction in CTSs with a live cell imaging system (Motion Vector Prediction; Sony, Tokyo, Japan). Upon treatment of an IKr channel blocker, E-4031, prolongation of field potential duration, which corresponds to QT-prolongation in electrocardiogram, was observed. Furthermore, CTSs exhibited reentrant tachyarrhythmias not only as a typical polymorphic EFP pattern but also as the reentrant spiral wave propagation accompanied by meandering of the waveform center which is a characteristic of TdP. The induction rate of TdP-like waveform in cell sheets with the mixed cell populations was 80% (12/15), whereas that in cell sheets with cardiomyocytes alone was 0% (0/18) ($p < 0.001$). The TdP-like waveform induction rate in 2D culture condition with the mixed cell populations was only 30% (3/10) ($p < 0.05$). These results indicate that the heterogeneity of the cellular components and the multi-layered 3D structure are both essential factors for reproducing TdP-like arrhythmia in vitro. Thus, we succeeded in inducing and visualizing TdP-like arrhythmia in our novel in vitro model using entirely stem cell-derived human heart tissue. This model would broadly contribute to provide novel understanding and analyzing method ofTdP as actual tissue behaviour by 3D in vitro modelling.

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W-1030

IN VITRO DRUG SCREENING ON FABRY DISEASE MODEL WITH CRISPR/CAS9-MEDIATED GLA GENE KNOCKOUT

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The CRISPR / Cas9 gene editing system is a powerful tool that can be used to generate mutations, deletions and corrections of genes in human cells. However, how to apply this tool to Fabry disease (FD) is worth exploring. Enzyme replacement therapy (ERT), a regular administration of recombinant human alpha Gal A (rhα-GLA), is a currently available and effective treatment to clear the accumulated GL-3 in FD patients. However, the rhα-GLA is physiologically instable and quickly degraded in cells. Moreover, lack of an appropriate in vitro disease model restricted the high throughput drug-screening for improving ERT efficacy. In addition, the lack of appropriate in vitro disease model is used to carry out studies to improve enzyme replacement therapy. Therefore, it is worth to establish a in vitro model of FD for screening potential candidates that can enhance and prolong ERT potency or half-life. In 293Tcells, knockout of the GLA gene using CRISPR / Cas9 technology resulted in GLA-null cells with a clear background of GLA and used to study the pharmacokinetics of rhα-GLA cells. The administrated rhα-GLA was decreased with time and had a half-life of 24 hrs in the GLA-null cells; co-administration of MG132 and rhα-GLA significantly restored the GLA enzyme activity by two-folds compared with rhα-GLA alone. In addition, co-treatment with rhα-GLA / MG132 in patient-derived fibroblasts resulted in an increase in GL-3 clearance of 30% compared to rhα-GLA alone. Collectively, CRISPR / Cas9-mediated GLA knock-out of HEK293T cells can be used to assess the intracellular pharmacokinetics of rhα-GLA as well as in vitro FD models for screening for prolonging rhα-GLA potency. To shed light on the direction of enhancing ERT efficacy in FD treatment, we established an effective in vitro model and demonstrated that co-treatment of MG132 prolonged the half-life of rhα-GLA and enhanced the clearance of GL-3.

W-1032

CRISPR CAS9-BASED TARGETING OF FLUORESCENT REPORTERS TO HUMAN IPSCS TO ISOLATE ATRIAL AND VENTRICULAR-SPECIFIC CARDIOMYOCYTES

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The discovery of induced pluripotent stem cell (iPSC) and CRISPR/Cas9 technologies has made it possible to investigate cardiovascular diseases with increased efficiency. Human iPSC-derived cardiomyocytes (hiPSC-CMs) have demonstrated significant promise for drug screening, disease modeling, and regenerative medicine applications. However, current approaches to generate hiPSC-CMs lead to a heterogeneous cell mixture containing atrial, ventricular, and nodal cells, limiting the accuracy and consistency of their read out. Here, using the CRISPR/Cas9 technology, we demonstrated the development of ventricular and atrial-specific fluorescent reporter lines by targeting the highly conserved chamber-specific genes MYL2 and SLN, respectively. We showed high efficiency insertion of MYL2-tdTomato (78%) and SLN-eGFP (86%) targeting cassettes into the expected genome loci following nucleofection and antibiotic selection. The MYL2-targeted hiPSC reporter lines expressed tdTomato in 57.8% of beating cardiomyocytes. By qPCR analysis, MYL2-tdTomato hiPSC reporter lines showed downregulated expression of pluripotent markers such as Nanog and Oct-4 and progressively increasing expression of cardiac genes such as cTNT, cardiac actin, and MYL2 over time. Immunohistochemical analysis confirmed the specificity of tdTomato expression in MYL2-expressing cardiomyocytes. Furthermore, single cell-based assessment of electrophysiological characteristics by patch clamp recordings validated the ventricular phenotype of the dTomato+ cells while traction force microscopy showed increased contractile force. The availability of a reliable method to isolate atrial and ventricular-specific cardiomyocytes from healthy and patient-derived hiPSCs should significantly improve the consistency and reliability of hiPSC-CMs for drug screening and disease modeling.

W-1034

EFFICIENT PRODUCTION AND PROCESSING OF CARDIOMYOCYTES FROM HUMAN PLURIPOTENT STEM CELLS USING STEMDIFF™ CARDIOMYOCYTE PRODUCTS

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Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) are used for disease modelling, drug discovery, and toxicology screening. However, the efficiency of producing hPSC-CMs is variable and, once established in culture, processing of hPSC-CMs for downstream assays is cumbersome. To overcome these limitations, we developed a complete workflow comprising several novel products: 1) STEMdiff™ Cardiomyocyte Differentiation Kit, 2) STEMdiff™ Cardiomyocyte Maintenance Kit, 3) STEMdiff™ Cardiomyocyte Dissociation Kit, 4) STEMdiff™ Cardiomyocyte Freezing Medium, and 5) STEMdiff™ Cardiomyocyte Support Medium. The STEMdiff™ Cardiomyocyte products are compatible with TeSR(TM)-E8(TM) and mTeSR(TM)1 media. The STEMdiff™ Cardiomyocyte Differentiation Kit produces a confluent beating monolayer of hPSC-CMs at day 15 of the differentiation protocol. More than 80% of the cells express cardiac Troponin T with a yield of >1x106 cardiomyocytes per well from 12-well plates seeded with all hPSC lines tested. The STEMdiff™ Cardiomyocyte Maintenance Kit maintains the health, quality and excitability of the hPSC-CMs for at least 1 month. Once the confluent beating monolayer of cardiomyocytes is established, cultures can be quickly and easily dissociated into single cells using the STEMdiff™ Cardiomyocyte Dissociation Kit, producing >80% viable cardiomyocytes. The dissociated single cardiomyocytes can be safely cryopreserved using STEMdiff™ Cardiomyocyte Freezing Medium. The cryopreserved cardiomyocytes can then be successfully thawed and replated using STEMdiff™ Cardiomyocyte Support Medium, producing >75% viable cardiomyocytes. In summary, the STEMdiff™ Cardiomyocyte products facilitate the robust production and maintenance of hPSC-CMs, and their processing to yield high quality, viable cells during dissociation, harvesting, cryopreservation, and re-plating. Such hPSC-CMs can then be used for a variety of downstream applications including FACS, immunocytochemistry, and electrophysiology.

W-1036

APPLYING IPSC TECHNOLOGY, NANOTECHNOLOGY, AND BIOINFORMATICS IN BIOMIMIC MODELS OF CARDIOMYOCYTE INJURY AND RESTORED THERAPEUTIC CONTRACTILITY

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Heart disease remains prevalent across the world as the number one killer spanning diverse populations. Stem cell based cardiomyocyte therapies have shown potential to play a critical role in recovery from myocardial infarctions directed at improving short and long-term outcomes of heart disease. However, cardiomyocyte therapeutic cells from multiple sources of adult, embryonic, and induced cells show differences in clinical trials that requires a more detailed understanding of the derived cardiomyocytes and their ability to integrate and direct changes. By use of transcriptomics, epigenomics, and bioinformatics analysis coupled with biomarker and functional analysis we previously evaluated replicate lines of ethnically diverse iPSC-derived cardiomyocytes. That study provided a first step using diverse donor tissues to generate cardiomyocytes and evaluate differences in differentiation pathways critical for function. Towards advancing cell therapeutic goals for cardiac health that use diverse iPSC derived cardiomyocytes we are now using these iPSC lines in combination with nanotechnology to establish a 3D model of infarction and repair in a dish to understand cardiomyocyte grafting and electromechanical coupling. Hypoxic damage is induced chemically by CoCl2 and by hypoxic chambers for comparison. By multifactorial analysis we evaluate cell biological parameters, including functional biomarkers, adherens and gap junctions, and cardiomyocyte or fibroblast characters to gauge electromechanical coupling with repairing cardiomyocytes. We are also tracking gene expression in GO pathways previously identified in our study and in others as relevant to successful contractility. The longterm goal of this work is to establish a reliable in vitro model for evaluation of cell therapeutic potential of cardiomyocytes. Such models could facilitate drug screening as well as expand our understanding of timelines, biomarkers and cell-integration/scarring and innervation. Such a detailed understanding is expected to be vital to advance and optimize future cardiomyocyte based therapies.

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ENDOTHELIAL CELLS AND HEMANGIOBLASTS

W-1038

BST2 ENHANCED OEC AND EC ADHESION IN ACUTE VASCULAR INJURY

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Outgrowth endothelial cells (OECs), the subtype of circulating endothelial progenitor cells (EPCs), home to injured vessel, initiating blood vessel regeneration. This process requires OECs initial adhesion to endothelial cells within the wounded site. Since its characteristic circulatory nature, injecting isolated OECs display troubling issues that injected OECs disperse through the blood vessel. In this study, we investigated IFN-r treated OECs enhanced wound healing through BST2-induced adhesion to endothelial cells. We found that IFN-r significantly up-regulated BST2 expression in both OECs and ECs. Up-regulated BST2 enhanced OECs to ECs interaction. However, IFN-r induced BST2 did not affect OECs adhesion independently. We found out BST2 increased OECs adhesion to ECs through tight homophiliac interaction of its extracellular domain (ECD). Treatment of BST2 ECD prior to OECs to ECs interaction decreased OECs adhesion to ECs. To find out whether there is adversary effect of treating IFN-r to OECs, we examined angiogenesis, migration and proliferation after treating IFN-r. We found out there was no effect of treating IFN-r to isolated OECs. Finally we injected IFN-r treated OECs to the wounded tail vein. We found out superior therapeutic effects on wound closing than injecting OECs alone or treating with BST2 ECDs to OECs prior to injection. Our data provide useful tool to enhance OECs adhesion to promote vessel regeneration and wound closing.

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W-1040

ROLE OF CXCR2 IN N-ACETYLATED PROLINE-GLYCINE-PROLINE (AC-PGP)-INDUCED ENDOTHELIAL PROGENITOR CELLS MOBILIZATION AND VASCULAR REGENERATION DURING THE REPAIR OF HINDLIMB ISCHEMIA

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Various therapeutic approaches to treat ischemic diseases using endothelial progenitor cells (EPCs) have been developed, in which EPCs integrate into blood vessels or stimulate neovascularization in the ischemic tissues. Therefore, mobilization and recruitment of bone marrow (BM)-derived EPCs are critical for de novo repair of ischemic tissue with neovascularization. Thus, the improvement in mobilization and recruitment of EPCs to sites of ischemic injury will enhance the therapeutic potential of EPCs and the regeneration of damaged tissues. Chemokine receptor 2 (CXCR2), a receptor of interleukin 8 (IL-8), mediates .inflammatory cell migration and angiogenic responses. We hypothesized that CXCR2 is involved in the mobilization and recruitment of EPCs from BM to the site of ischemic injury for tissue protection via neovascularization. We evaluated the role of CXCR2 in angiogenesis and tissue regeneration by stimulating EPCs with Acetylated Pro-Gly-Pro (Ac-PGP), which is the endogenous degradation product of extracellular collagen and binds to CXCR2. Intramuscular injection of Ac-PGP at ischemic hindlimb promoted blood perfusion, neovascularization, and limb salvage. The mobilization of Flk-1+ Sca-1+ EPCs in peripheral blood increased after intramuscular injection of Ac-PGP, which peaked on day 3 after ischemia induction. Furthermore, in chimeric mice in which BM was reconstituted from GFP mice, Ac-PGP stimulated recruitment of BM-derived EPCs in ischemic hindlimb and incorporation of GFP-positive cells to newly-generated ILB4- and α-SMA-positive blood vessels, which verifies the contribution of BM-derived EPC to neovascularization during the repair of ischemic hindlimb. In CXCR2 knockout mice, Ac-PGP-induced EPC mobilization, de novo neovascularization, and ischemic hindlimb salvage were significantly declined. These results suggest that Ac-PGP has a therapeutic effect by stimulating neovascularization through mobilization of BM-derived EPCs via CXCR2-dependent mechanism.

HEMATOPOIESIS/IMMUNOLOGY

W-1042

GENERATION OF MONOCYTE DERIVED DENDRITIC CELLS USING XENO-FREE CLINICAL GRADE HUMAN PLATELET LYSATE

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Dendritic cells (DCs), a major antigen-presenting cell type, have been used widely for vaccine development in cancer immunotherapy. Among all the sources of DCs, those derived from CD14+ monocytes (Mo-DCs) are favored for therapeutic use because they: 1) have strong T cell immunity inducing ability, 2) are relatively easy to generate, and 3) do not require cytokine pretreatment. Clinical manufacturing of Mo-DCs has traditionally relied on the use of either fetal bovine serum (FBS) which poses potential risks for viral and prion transmission as well as for adverse immunological reactions, or human AB serum (hABS) which is collected from a small number of donors and has considerable lot-to-lot variability. To address these issues, we have developed a method for Mo-DC production using a xeno-free cell culture supplement: PLUSTM human platelet lysate. PLUSTM is manufactured in large lots under Good Manufacturing Practice (GMP) standards using platelet units obtained from AABB-accredited blood banks. We undertook this study to characterize the maturation state and functionality of PLUSTM-generated Mo-DCs in comparison to those generated using FBS or hABS. To perform the experiments, freshly isolated CD14+ monocytes (n=3) were differentiated into immature Mo-DCs by 4-days incubation in RPMI 1640 containing IL-4 (400 U/mL), GM-CSF (1000 U/mL) and either 5-10% FBS, 2.5-5% hABS, or 2.5-5% PLUS™, followed by 2-days maturation in FBS, hABS, or PLUSTM supplemented media with either TNF-α, IFN-γ or lipopolysaccharide. Flow cytometry analysis demonstrated that PLUSTM-generated Mo-DCs had a mature phenotype, with equivalently negative CD14 expression and equivalently positive CD209, CD83, CD86 and HLA-DR expression as FBS and hABS generated Mo-DCs. Phagocytosis of FITC-dextran was similar for Mo-DCs generated under all culture conditions. In a 6 day co-culture study, PLUS™-generated Mo-DCs stimulated allogeneic T cell proliferation as efficiently as FBS and hABS. Antigen expressions, dextran phagocytosis, and T cell stimulation were consistent between all maturation pathways and were independent of FBS, hABS, or PLUSTM concentration. These studies demonstrate that PLUS™ human platelet lysate can successfully replace

FBS or hABS for ex vivo production of clinical-grade Mo-DCs for immunotherapy applications.

W-1044

A HUMAN IPS MODEL IMPLICATES EMBRYONIC B-MYELOID FATE RESTRICTION AS A DEVELOPMENTAL SUSCEPTIBILITY TO ETV6-RUNX1

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Childhood acute lymphoblastic leukemia (cALL) is clinically distinct from that in adults with a higher incidence, better prognosis and distinct mutational spectrum. One hypothesis for this difference is that cALL arises in transient cells unique to early human development. We explored this in ETV6-RUNX1 cALL where evidence from twins and neonatal heel prick testing has shown that this mutation arises in utero and is an initiating event. We characterized B-lymphoid development in first trimester human embryos aiming to identify a transient compartment vulnerable to the pre-leukemic initiating effect of ETV6-RUNX1, the most common mutation in cALL. The first B cells emerging in human ontogeny uniformly express surface IL7 receptor (IL7R) and appear to derive from a CD19 negative IL7R+ progenitor compartment. The IL7R+ progenitor has B and myeloid potential in vitro and single cell qPCR analysis revealed a transition during development from a primarily myeloid to a predominantly lymphoid state, transiting through a B-myeloid state that was rare or not present at other developmental stages investigated. In vitro B cell differentiation of human pluripotent stem cells (hPSCs) recapitulates these features. Global gene expression analysis further supports that in vitro differentiation of hPSCs closely resembles what is seen during human development, thereby providing a tractable and developmentally relevant model in which to study pre-leukemic initiation of cALL. hPSCs were genomic engineered to express ETV6-RUNX1 from the endogenous ETV6 locus. Differentiation of ETV6-RUNX1+ hPSCs demonstrated impairment of B lineage commitment from the IL7R+ compartment, blocking differentiation and producing proB cells that aberrantly co-express B and myeloid genes and that retain myeloid potential. We propose that the lineage dynamics of the fetal IL7R+ compartment are particularly susceptible to dysregulation by ETV6-RUNX1, thereby providing an explanation for the childhood restriction of cALL.

Funding Source: Wellcome trust Research Training Fellowship, UK NIHR Academic Clinical Fellowship, Swedish Childhood Cancer Foundation, Swedish Cancer Society, Crafoord foundation, Hemato-Linné Grant, Tobias Foundation and Bloodwise.

W-1046

REAL-TIME PLATELET PRODUCTION FROM HUMAN IPS-DERIVED MEGAKARYOCYTES IN A MICROPILLAR-TEXTURED MICROFLUIDIC CHIP

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Bioreactors that recapitulate some features of the bone marrow microenvironment and cell reprogramming techniques to produce megakaryocytes (MKs) represent major improvements towards ex vivo platelet generation for therapeutic applications to treat or prevent bleeding in severe thrombocytopenic patients. In this study, we provide evidence of efficient production of platelets, based on a new microfluidic chip that upregulates and synchronizes platelet production by exposure of MKs to von Willebrand Factor (VWF) at a high shear rate. Mature MKs are directly injected into microchannels comprising arrays of VWF-coated pillars acting as anchors. Captured MKs adopt a beads-on-a-thread conformation before being fragmented into proplatelets and releasing platelets in the flow of the perfusion. MKs derived from hUES cells and iPS cells, as well as from primary hematopoietic stem cells (HSC), including cord blood and peripheral blood, underwent similar sequences of fragmentation into proplatelets and platelets, indicating an efficient and robust process. Time-lapse observation allowed measuring single MK elongation velocity, as well as number of releasable platelets per MK, calculated as ratio of released bead volume / smallest volume of released element defined as a platelet. In each of the 4 experiments, mean elongation velocities of MKs obtained from hUES cells and iPS cells were calculated and varied between 23.7 and 48.1 µm/min and 30.0 and 57.3 µm/min, respectively. These values were slightly higher than those of 19 µm/min previously reported using iPS-derived MK in another platelet bioreactor. The number of releasable platelets per MK varied between 20 and 800, and 39 and 833, for MK derived from hUES cells and iPS cells, respectively. Newly formed platelets were collected for characterization showing specific hallmarks of platelet such as CD42b expression and the presence of a tubulin ring. In conclusion, these data indicate that high numbers of platelets are releasable from iPS cell-

derived MK anchored onto VWF-coated micropillars at high shear rates and provide an innovative way to overcome the limitations of low platelet shedding from iPS cell-derived MKs in static conditions.

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W-1048

EXPANSION OF HSCS FROM CORD BLOOD CD34+ CELLS BY VALPROIC ACID REQUIRES BOTH MITOCHONDRIAL REMODELING AND P53

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The use of Umbilical Cord Blood (UCB) as a graft for adults has been restricted due to the limited numbers of HSCs. We have previously reported that UCB CD34+ cell number can be expanded by ex vivo treatment with a cytokine combination and the histone deacetylase inhibitor, valproic acid (VPA). We show that VPA triggered two distinct phases of HSC behavior. The first phase was characterized by a slight increase in the numbers of HSCs due to expression of pluripotency genes. The second phase was characterized by a greater number of cell divisions and loss of quiescence. These events resulted in a drastic increase in the number of phenotypically defined HSCs. VPA-induced HSCs maintained a mitochondrial profile characterized by low mitochondrial potential, ROS levels and mass. The low metabolic profile was correlated with a primitive mitochondrial network comprised of cristae-poor mitochondria. Notably, these events are reversible and do not result in the malignant transformation of the CD34+ cells. Removal of VPA from the culture after 24, 48 and 72hrs of incubation suppressed the HSC expansion and increased mitochondrial ROS, mass and potential. Thus, the epigenetic reprograming and mitochondrial remodeling triggered by VPA were tightly linked to fuel dedifferentiation of CD34+ cells into HSCs. Besides remodeling the mitochondrial network, the ex-vivo expanded HSCs transiently upregulated p53. P53 inhibition by siRNA and pifithrin-α led to a significant reduction of the HSC pool induced by VPA. The diminished expansion was correlated with a significant elevation of the mitochondrial ROS, but not the mitochondrial mass. P53 inhibition suppressed MnSOD and sestrin2, both critical for the antioxidant defense mechanism. Conversely, co-treatment with VPA and anti-oxidant NAC or nutlin (1μM), an inhibitor of p53 degradation decreased further mitochondrial ROS levels and augmented the expansion of HSCs. Given that ROS limit the lifespan and long-term characteristics of HSCs, our findings suggest that the VPA-expanded HSCs are equipped with mechanisms that monitor and ensure their fitness. Therefore, VPA triggers both mitochondrial remodeling and p53 activation to suppress ROS and metabolically support the epigenetic modifications leading to expansion of HSCs from UCB CD34+ cells.

Funding Source: NYSTEM

W-1050

DIFFERENTIAL IMMUNOGENICITY OF HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSC) IS DETECTABLE IN HUMANIZED NSG MICE WITH AUTOLOGOUS IMMUNE SYSTEM

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Different cell lineages derived from autologous human induced pluripotent stem cells (hiPSC) would offer great advantages in transplantation medicine, as this procedure can bypass the major problems with transplantation, such as graft versus host (GVH) reaction, tissue rejection, and immunosuppression. To further validate the prospective utility of hiPSC for clinical use, pre-clinical evaluation of both differentiated and undifferentiated hiPSC in appropriate animal models is essential. We used a humanized mouse model reconstituted with autologous human immune system to test the immune compatibility of ex vivo-engineered hiPSC. NOD/scid-IL-2Rg null (NSG) mice were injected with cord blood-derived CD34+ hematopoietic stem cells (HSCs), which develop into functional human immune system. Umbilical cord tissue-derived fibroblasts were used for hiPSC generation and pluripotency was confirmed using flow-cytometry, immunocytochemistry and RT-PCR. For the teratoma assay, hiPSC were injected into humanized mice with both autologous and allogeneic human immune system. Non-humanized NSG mice injected with hiPSC were also included as controls to compare teratoma growth. Mice were euthanized 7-8 weeks post hiPSC injection to harvest teratoma and analyzed for immune cell infiltration by immunohistochemistry. Blood and spleen were also collected to determine immune activation by flow-cytometry. All mice injected with hiPSC developed well-characterized (with ectoderm, endoderm and mesoderm germ layers) teratomas ranging from 1-2 cm in size. Significant infiltration of cytotoxic T (CD8+) cells was observed in teratomas generated from allogeneic hiPSC with concomitant increase in T cell (CD8+, HLA DR+) activation. All iPSC-injected mice showed human

immune cell activation when compared to humanized mice without iPSC. However, mice with allogeneic hiPSC displayed increased memory T cells (CD45RO+, CCR7+ and CD45RO+, CD62L+), pro-inflammatory (intermediate or non-classical CD14+, CD16+) monocytes, and activated cytotoxic T-cells (CD8+, HLA DR+) when compared to autologous hiPSC injected mice. Hence, ex vivo engineered hiPSC derived tissue immunogenicity can be evaluated in the humanized mouse model for autogenic and allogeneic immune reactions before therapeutic use in human.

W-1052

INHIBITION OF ALDEHYDE DEHYDROGENASE EXPANDS HUMAN HEMATOPOIETIC PROGENITOR CELLS WITH VASCULAR REGENERATIVE FUNCTIONS

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The transplantation of blood-derived progenitor cells holds clinical potential for the treatment of severe diabetic comorbidities, such as critical limb ischemia. Umbilical cord blood (UCB)-derived hematopoietic progenitor cells (HPC) initially purified using high aldehyde dehydrogenase (ALDH) activity demonstrate potent pro-angiogenic functions in NOD/SCID mice with unilateral hind limb ischemia. However, UCB ALDHhi cells are extremely rare and fail to retain vascular regenerative functions when expanded beyond 6 days. ALDH is the rate-limiting enzyme involved with the intracellular production of retinoic acid (RA), a potent driver of HPC differentiation via the RAR/RXR complex. Thus, expansion of UCB ALDHhi cells represents a paradoxical challenge to maintain proliferation while limiting differentiation. We sought to determine if inhibition of RA-production would delay differentiation of UCB ALDHhi cells ex vivo, and prevent the loss of vascular regenerative function after transplantation. Human UCB ALDHhi cells were cultured under serum-free conditions (X-vivo 15 + SCF, TPO, FLT-3L) for 9 days, with or without the reversible ALDH-inhibitor diethylaminobenzaldehyde (DEAB). Although total cell numbers were increased >70-fold after 9 days expansion, CD34+/CD133+ expression and high ALDH-activity were severely diminished under basal conditions. In contrast, DEAB-inhibition increased the total number of cells retaining high ALDH-activity and primitive surface marker expression by 20-fold. DEAB supplementation enhanced the myeloid multipotentcy of day 9 HPC coinciding with reduced differentiation towards the megakaryocyte lineage (CD41+/CD42+/CD38+). RT-PCR confirmed DEAB inhibition reduced RA-signaling within HPC. Comparative proteomic analysis revealed HPC expanded under DEAB-conditions upregulated anti-apoptotic proteins and diminished the production of extracellular matrix proteins implicated in platelet coagulation. Intramuscular transplantation of DEAB-treated HPC promoted the recovery of hind-limb perfusion by stimulating murine endothelial cell proliferation in vivo. Collectively, we demonstrate inhibition of RA-production delays HPC differentiation towards the megakaryocyte lineage and expands early myeloid HPC with pro-angiogenic functions.

Funding Source: Hear and Stroke Foundation of Canada, Juvenile Diabetes Research Foundation

W-1054

INHIBITION OF RECEPTOR PROTEIN TYROSINE PHOSPHATASE-SIGMA (PTPΣ) PROMOTES HEMATOPOIETIC STEM CELL (HSC) REGENERATION

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Receptor tyrosine kinases, such as c-kit, Flt-3 and Tie2, regulate hematopoietic stem cell (HSC) proliferation, differentiation and maintenance. Substantially less is known about the function of receptor protein tyrosine phosphatases in HSCs. We discovered the receptor protein tyrosine phosphatase sigma (PTPσ) is highly expressed by murine and human HSCs. Constitutive deletion of PTPσ caused a marked increase in HSC repopulating capacity as measured in primary and secondary competitive repopulation assays (Quarmyne et al. JCI. 125:177-182, 2015). Negative selection for PTPσ expression also conferred a 10 fold increase in the engraftment of human cord blood cells in mice. Here, we sought to develop a pharmacologic strategy to inhibit PTPσ function as a means to augment HSC functional capacity. Via a small molecule screen, we identified small molecule 5483071 (Chembridge) as a candidate PTPσ inhibitor. In silico simulation indicated that 5483071 (3071) rigidly docked into the binding site of PTPσ intracellular domain through hydrogen bonding and electrostatic interactions. Subcutaneous administration of 3071 to irradiated (750 cGy) C57BL/6 mice for 10 days conferred 100% survival compared to 40% survival in control mice (p=0.0007). 3071 treated mice displayed accelerated recovery of white blood cells (P=0.002), neutrophils (P=0.02), HSCs (P=0.02), and bone marrow (BM) colony forming cells (P=0.001) compared to saline treated, irradiated controls. In a model of bone marrow transplantation, treatment with 3071 also significantly increased the survival of mice transplanted with a

limiting dose of 1 x 10^5 BM cells compared to identically transplanted mice treated with saline (P=0.03) and significantly increased donor hematopoietic cell engraftment (P=0.003). Mechanistically, treatment with 3071 decreased HSC apoptosis after irradiation (P=0.02) and caused the activation of the RhoGTPase, Rac1, in HSCs (P=0.0004). Importantly, treatment of BM Lin- cells from PTPσ -/- mice with 3071 caused no effect on Rac1 activation or HSC regeneration, suggesting that 3071 binds specifically to PTPσ. Inhibition of PTPσ promotes hematopoietic regeneration and augments hematopoietic reconstitution following transplantation, suggesting the potential of PTPσ inhibitors for regenerative medicine in hematology.

W-1056

INTEGRATION-FREE SYSTEM FOR GENERATION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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The generation of hematopoietic stem cells (HSCs) from human pluripotent stem cells (PSCs) constitutes a valuable tool with promising applications for research and therapy. However, derivation of HSCs with in vivo long-term engraftment and multi-lineage potential remains elusive. We have described a combinatorial approach, based on the directed differentiation of hemogenic endothelium (HE) and transduction with five transcription factors (TF) (RUNX1, ERG, LCOR, HOXA5 and HOXA9) expressed in lentiviral vectors that allowed the conversion of human PSCs into hematopoietic stem and progenitor cells (HSPCs). The resulted cells exhibited long-term and multi-lineage hematopoietic capabilities when injected into irradiated immune-deficient mice. Despite this proof of principle, the engineered cells have a limited self-renewal capacity due to the integration of the transgenes and are still molecularly distinct from bona fide HSCs. Thus, in an attempt to achieve bona fide HSCs and make them safer for future therapeutic interventions, we have established integration-free systems that have shown comparable efficiency to the previously developed lentiviral strategy through in vitro and in vivo experiments. Therefore, this new method may overcome some limitations of the lentiviral approach and hold the key for future regenerative medicine advances in blood diseases.

W-1058

IPSC-BASED MAPPING OF GLOBIN EXPRESSION THROUGHOUT HUMAN HEMATOPOIETIC DEVELOPMENT

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It has recently become clear that in vitro hematopoietic differentiation protocols using pluripotent stem cells capture the primitive wave of hematopoietic development with limited induction of the definitive wave that gives rise to cells with adult-type characteristics such as the ability to express adult β-globin. Recent efforts from our group to derive more definitively patterned erythroid cells from induced pluripotent stem cells (iPSCs) have resulted in a significant increase in β-globin transcripts. To better quantify our progress in augmenting β-globin expression and to track globin ontogeny in real-time, we have created a β-globin reporter iPSC line that allows for the mapping of globin expression throughout erythropoietic development. To create this tool, a promoterless GFP cassette was fused in frame to the first codon of the β-globin gene allowing for visualization of β-globin expression at single cell resolution by looking at GFP expression via FACS. Whereas a clear increase in β-globin transcripts is seen by qRT-PCR as the cells progress through differentiation, only about 1% of cells exceed the GFP detection threshold at the most mature stage of differentiation, suggesting a discrepancy between RNA level and protein level expression of β-globin in our iPSC-based system. The creation of a tool to distinguish live cells expressing β-globin at the protein level allows us to interrogate both negative and positive populations via single cell RNA sequencing and gain insight into this observed discordance between transcriptional and protein level expression. At the same time, this approach is expected to result in a signature for β-globin expressing cells that will be instructive for future strategies aimed at increasing β-globin protein levels in iPSC-derived erythroid cells to improve the resolution with which we can study hemoglobinopathies such as β-thalassemia and sickle cell disease. Such strategies can then immediately be tested using the β-globin reporter iPSC line as a screening platform.

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W-1060

A SPECIFIC CHEMICAL AND TRANSCRIPTION FACTOR COMBINATION SPECIFIES AND EXPANDS HEMATOPOIETIC STEM AND PROGENITOR CELLS IN ZEBRAFISH EMBRYONIC CELL CULTURE

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Advances in transcription factor reprogramming and directed differentiation strategies have brought us tantalizingly close to generating HSCs for regenerative medicine, but these cells are embryonic in nature and lack the essential properties of true, definitive blood stem cells. To identify new chemical and genetic ways to specify and expand HSCs, we developed a zebrafish embryo culture system in which we can examine activation of specific blood and endothelial markers. Fluorescent transgenic zebrafish embryos are dissociated into single cells prior to hemangioblast development at 9 hours post fertilization (hpf), or prior to HSC birth at 24 hpf, then plated with chemicals. This system is similar to iPSC differentiation, but tissues form in just 1 to 2 days. A high-content automated imager reads out fluorescence from the transgene after 24 or 48 hours in culture. We screened 3,840 compounds using sclα:dsRed/sclβ:GFP embryos, which delineate endothelial cells that are about to bud into HSCs. Inhibition of Rho-associated kinase (ROCK) signaling and the TGF-β pathway caused expansion of these cells, indicating differentiation of hemogenic endothelium that can ultimately give rise to HSCs. A methyltransferase inhibitor and an endocannabinoid receptor agonist also expanded sclα/sclβ cells. However, none of these chemicals were able to activate expression of the specific HSC marker runx1:GFP, which has been notoriously difficult to switch on ex vivo. To overcome this barrier, we sought to identify transcriptional regulators of HSC development. We previously showed that ERG, HOXA9, MYB, RORA and SOX4 reprogram iPSC derived hemogenic cells to a transplantable fate. In vivo studies showed that injection of these factors into transgenic cd41:GFP embryos leads to expansion of HSPCs by 44 hpf. When injected embryos were dissociated at 24 hpf and plated in our culture assay, we observed an increase in both cd41:GFP and runx1:GFP cells. Our studies have identified a combination of transcription factors and small molecules that switch on runx1 to drive differentiation of HSPCs from early cultured hemogenic endothelial cells, which could not be achieved with chemicals alone. This will provide new insight towards the ultimate goal of generating patient-specific HSCs from iPSCs for the treatment of blood diseases and cancers.

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PANCREAS, LIVER, KIDNEY

W-1062

DOE BASED STAGE-WISE OPTIMIZATION FOR DIRECTING THE DIFFERENTIATION OF DORSAL PANCREAS

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During the onset of organogenesis the pancreas buds from two spatially distinct regions occurring on the dorsal and ventral side of the gut tube. Although these two pancreatic buds eventually merge there are distinguishing characteristics between them including differences in gene expression as well as their final contributions to the tail and the head of the pancreas. Substantial effort is given to direct the differentiation of pluripotent cells towards pancreatic lineages with the objective of generating insulin producing cells; however, this work is done with little consideration to the identity of the pancreatic progenitor created. Using a systems developmental biology approach resting on high-dimensional experimental designs we have investigated differentiation of pluripotent cells to the pancreatic state. This method is unbiased and highly predictive resting on mathematical modeling of key fate determining genes. By following induction of endodermal and pancreatic genes we identify a novel induction method for definitive endoderm (DE) patterned to an anterior dorsal endodermal (ADE) state. Comparing this approach for endoderm induction to using Activin/Wnt to induce DE (literature standard) it is noted that both methods activate core endoderm genes including EPCAM, CXCR4, FOXA2, FOXA3 and GATA6, however, the ADE protocol is shown to have elevated levels of anterior endoderm genes SOX2 and SFRP5. Furthermore, ADE induction results in higher expression of MNX1, PTCH1, NOGGIN, NEUROD and PAX6 all genes known to be enriched in dorsal pancreatic bud. In contrast the DE generated using literature standard is shown to have a higher expression of the posterior/ventral gene SOX17 and the ventral-specific genes HHEX and NR5A2. We believe that literature standard protocols generate a ventral DE population (VDE), with more posterior

aspects including stray fate opportunities to Liver and intestine. In this work, we noted that both VDE and ADE protocols generate pancreas (PDX1) with comparable efficiency, and downstream competence for endocrine, ductal, and acinar differentiation. Altogether we demonstrate a novel approach for generating dorsal endoderm that in turn has pancreatic potential, arguing that as in the normal embryo, there exist two distinct pathways for creating pancreas from pluripotency.

W-1064

SCALABLE GENERATION OF FUNCTIONAL AND TRANSPLANTABLE HEPATIC CELLS FROM HUMAN ENDODERM STEM CELLS

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The ability to generate in large scale the functional and transplantable hepatic cells, including hepatocytes and cholangiocytes, is critical for cell-based therapy for the treatment of liver diseases. The in vitro differentiation system of Pluripotent Stem Cells (PSCs) that allows the efficient generation of functionally mature hepatic cells in large quantities is still missing. Also, the use of PSC-derived hepatic cells for cell-based therapies is undermined by the teratoma-forming potential of any residual undifferentiated cells in the system. The self-renewable human Endoderm Stem Cells (EP Cells) that are derived from PSCs are non-tumorigenic in vivo and have robust hepatic differentiation capacity, and therefore can serve as better starting material than PSCs to prepare hepatic cells in large quantities for future clinical applications. Here we report the establishment of a three-dimensional suspension culture system that enables efficient large-scale generation (~10^9) of mature hepatocytes and cholangiocytes from EP cell within a month. The newly developed protocol yields polarized cystic structures that are important for the maturation and viability of hepatocytes. Two Signaling pathways have been identified in this study to play important roles in the maturation of hepatocytes or cholangiocytes. The gene expression profiling by single-cell transcriptomic analyses and the functionality assays reveal that the EP cell-derived hepatocytes and cholangiocytes are comparable to their siblings in the adult rather than fetal livers. When transplanted into the animals with FAH deficiency or DDC-induced liver injury, the EP cell-derived hepatocytes or cholangiocytes are able to repopulate the host livers. The liver repopulating efficiencies of the CYP3A4+Albumin+ cells and the CYP3A4-Albumin+ cells are being measured in non-human primate models. The establishment of the large-scale hepatic differentiation system of EP cells lays the foundations for the cell–based applications for liver diseases.

W-1066

MESENCHYMAL STEM CELLS TRANSFOR INTO MESANGIAL CELLS AS THEY REPAIR THE DAMAGED MESANGIUM

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Mesenchymal stem cells (MSCs) have been indicated that they only provide paracrine effects when engaged in the process of tissue repair. In order to assess the specific role of MSCs in mesangial repair, a combined model of in-vitro and in-vivo was used for the MSCs interaction with glomerulopathic light chains (G-LCs) induced mesangial cells (MCs) injury. A 6 dimensional (6D) live cell imaging system was the in-vitro system used. MCs were incubated with G-LCs obtained from the urine of patients with renal biopsy-proven AL-amyloidosis (AL-Am) and light chain deposition disease (LCDD). Similar amyloidogenic and light chain deposition-associated G-LCs were perfused through the renal artery in the ex-vivo platform. The respective lesions were reproduced in both platforms. Then, tagged MSCs were introduced. Immunofluorescence, immunohistochemistry and electron microscopy were used to evaluate samples obtained at different time frames. Stains for smoothelin, a smooth muscle specific actin; CD68, a microphage cell marker; CD29 and CD 54 two mesenchymal stem cell markers were used to monitor phenotypic transformation of MSCs in the process of repair. Ultrastructural morphology and activation by using electrical field stimulation to assess the ability of cells to contract was utilized as the method to assess functionality. MSCs transformed from an undifferentiated to a macrophage phenotype to clear the damaged mesangial areas. The process showed transformed MSCs phagocytosing cellular debris resulting from apoptotic mesangial cells and damaged matrix elements, and amyloid fibrils. After the cleaning process was finished, MSCs acquired morphologic, functional, and immunophenotypic characteristics of MCs as they proceeded to lay down new mesangial

matrix. MSCs manifest great plasticity as they proceed to repair the G-LCs damaged mesangium. The fact that they transform to a macrophage phenotype followed by transformation to MCs allows them to perform different crucial functions during the process of repair. The restored mesangium is possible as new MCs derived from MSCs are able to reproduce the normal mesangium and function as normal MCs.

W-1068

PANCREATIC DIFFERENTIATION FACTORS IDENTIFIED BY A NOVEL BIOINFORMATICS WORKFLOW

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Understanding how the human pancreas develops in vivo is essential for efforts in regenerative medicine to produce in vitro clinically relevant cell types for the treatment of pancreatic diseases, such as human pluripotent stem cell (hPSC)-derived, insulin-secreting beta cells for the treatment of diabetic patients. In an effort to decode some of the earliest molecular events during human pancreatic morphogenesis, we have combined numerous datasets, both published and unpublished, into a bioinformatics workflow that aims to elaborate the gene regulatory network (GRN) downstream of the atypical homeobox gene Pancreatic and Duodenal Homeobox 1 (PDX1). PDX1 is situated high atop the pancreatic GRN, as patients with inactivating mutations display pancreatic agenesis. Our workflow thus incorporates (1) PDX1 ChIP-Seq on hPSC-derived human pancreatic progenitors; (2) multi-organ RNA-Seq including the developing human pancreas from CS17-19 stage embryos; and (3) comparative microarrays between wild-type and PDX1-deficient cell populations that have been differentiated in parallel. From these analyses, we have shortlisted four genes for further study that we predict are direct transcriptional targets of PDX1—HES4, SEMA6A, SARM1 and HRK, all of whom are interestingly known to function during neuronal development. We independently validated the selection of these four by demonstrating that their expression kinetics closely follow that of PDX1 during pancreatic differentiation in vitro. Next, we intend to examine the roles of these genes during pancreatic differentiation, and this will be accomplished by engineering mutations in each gene with a high fidelity CRISPR-cas9 indel mutation approach in hPSCs. Once differentiated, we will identify the downstream DNA targets of HES4, for example, which is a human-specific transcription factor belonging to the HES family of transcriptional repressors and additionally perform comparative RNA-Seq between mutant and wild-type cells. HES proteins are known to heterodimerise, and we hypothesise that HES4 signals through the non-canonical NOTCH signalling pathway to repress HES1 and/or NGN3.

W-1070

AGE-LINKED CHANGES IN MURINE PERIVASCULAR CELLS DURING KIDNEY INJURY AND REPAIR

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The kidney is susceptible to ageing. Injuries in old patients more rapidly progress to end stage renal disease, and mouse models show increased injury in aged animals, yet the reasons for this are unknown. As in other organs, pericytes play a key role in the kidney's injury response, and a minority of pericytes expressing Gli-1, a sonic hedgehog (Shh) receptor, contribute the majority of renal myofibroblasts and fibrosis following injury in mice. We hypothesise that differences in pericyte subtypes between old and young organs can explain the age-linked deterioration in tissue repair. This project aimed to interrogate pericyte subpopulations using surface marker expression, to identify potential differences in the starting or responsive pool of cells between ages, and whether such differences are meaningful to the wound healing response, with the ultimate aim of defining beneficial/pathological pericyte subpopulations. The effects of Shh inhibitor Vismodegib during injury were also investigated. Pericytes were detected in baseline old and young kidneys using the markers platelet derived growth factor receptor β (PDGFR-β) and CD146, which together show differential expression across kidney regions. Old kidneys had more CD146 positivity in the outer stripe of the outer medulla (OuS). Old and young animals were subjected to unilateral ischemia reperfusion injury and experienced similar levels of tubular death in the OuS. However although both age groups had increased fibrosis in injured kidneys old animals also had increased fibrosis in contralateral uninjured kidneys. When PDGFR-β and -α were observed together, the PDGFR-α+ ratio increased in old animals. Other cellular differences detected include a population of CD45+ ICAM2+ cells exclusive to old kidneys. When treated with Vismodegib following a unilateral ureterial obstruction no difference in fibrosis was detected. Together these results report pericyte marker heterogeneity between kidney regions, and increased fibrosis in uninjured organs of old mice, but

no influence of Shh inhibition on myofibroblast activity. Further elucidating changes in cell populations of aged animals, which have increased susceptibility to injury and impaired recovery, should improve understanding of optimal repair processes and produce novel therapeutic targets.

Funding Source: This work was funded by the Medical Research Council (UK)'s Tissue Repair PhD program at the University of Edinburgh.

W-1072

A DESIGN-OF-EXPERIMENT APPROACH TOWARDS DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO DEFINITIVE KIDNEY CELL LINEAGES

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The kidney is a vital organ required for waste excretion as well as water and solute reabsorption. Its functionality relies on a range of highly specialized cells that are arranged into nephrons, the functional units of the kidney. While we understand many of the properties of the different renal cell types, we lack effective methods to generate large quantities of terminally differentiated human-derived kidney epithelial cells from human pluripotent stem cells. To address this critical knowledge gap we utilize Quality-by-Design-based methods, in particular the Design-of-Experiment (DoE) theory, to provide a systematic, data driven approach towards renal cell differentiation. In a single experiment we simultaneously test the effect of up to 12 morphogen inputs on more than 50 target genes. Experimental design and statistical methods provide an in-depth understanding of the input parameters. Moreover, compilation of a series of these experiments lead to a systems-developmental biology representation of the control logics underlying renal epithelial cell differentiation from pluripotent stem cells. Here we now report our progress for the generation of podocytes, a specialized renal epithelial cell type found in the glomerulus of the kidney. Starting from established protocols we differentiate human embryonic stem cells into nephric mesenchyme precursors. Using DoE we then identify culture conditions to differentiate these cells along the podocyte lineage in a stepwise protocol. These cells not only express transcription factors (e.g. WT1 or MAFB) as well as structural proteins (e.g. PODXL) characteristic for podocytes, but also exhibit structural and physiological properties of these highly specialized renal epithelial cells. Importantly, the conditions are highly robust and lead to the formation of a homogenous culture of human podocytes. Together, this study, to our knowledge, is the first of its kind identifying a protocol for the generation of stem cell-derived podocytes. It will provide a unique resource to understand podocyte biology and its disturbance during disease formation. The cells will also provide a valuable tool to develop and/or test clinically relevant therapies and devices towards treating the range of human kidney diseases characterized by podocyte dysfunction.

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EPITHELIAL TISSUES

W-1074

EPIDERMAL YAP ACTIVITY DRIVES ROCK SIGNALING AND DERMAL FIBROSIS TO PROMOTE EPIDERMAL STEM/PROGENITOR CELL PROLIFERATION IN THE MOUSE SKIN IN VIVO

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Yes-associated protein (YAP) is a pivotal regulator of stem cells that acts as a mechanosensor in the control of tissue regeneration. The molecular mechanisms that control stem cell proliferation in response to YAP activity remain unknown. We discovered that YAP activity in the mouse epidermis drives mechanotransduction pathways regulated by Rho kinase (ROCK), independently of epidermal β-catenin, to elevate collagen-mediated dermal stiffness. The dermal fibrosis causes increased integrin ligation and FAK activation, eventually resulting in increased β-catenin activity and epidermal stem/progenitor cell proliferation in vivo. Furthermore, we observed increased nuclear YAP and β-catenin localization with tumour progression in murine and in human cutaneous SCC, together with gradually increasing ROCK-dependent mechanosignalling and stromal fibrosis. Our work reveals that YAP acts as a central mechanoprotein capable of not only responding to but also driving mechanical force. This has implications for our understanding of normal tissue regeneration and the development of cancers displaying increased YAP activity.

Funding Source: National Health and Medical Research Council of Australia, UNSW Sydney, Australian Research Council, Cancer Council SA and the Health Services Charitable Gifts Board SA

W-1076

HIGH EXPRESSION LEVELS OF CD200 AND CD200R1 DISTINGUISH STEM AND PROGENITOR CELLS IN THE MAMMARY REPOPULATING UNITS

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Elucidating the top of the mammary epithelial cell hierarchy is highly important for understanding its regeneration capabilities and identifying target cells for transformation. Aiming to obtain an enriched mammary epithelial stem cell population, CD200highCD200R1high epithelial cells were identified. These cells represented ~50% of the CD49highCD24med mammary repopulating units (MRUs) and are termed here MRUCD200/CD200R1. Following transplantation into de-epithelialized mammary fat pads, a larger area was occupied by new outgrowths from these cells as compared to the rest of the MRUs, termed MRUnot CD200/CD200R1. Gene-expression profile of 1000 representative cells indicated that MRUCD200/CD200R1 maintains a much lower number of divergently defined highly expressed genes and metabolic pathways that support cellular movement, tissue development, cellular development and cell growth and differentiation, as well as progenitor activity than their MRUnot CD200/CD200R1 counterpart. The complement system was among the few highly expressed pathways in MRUCD200/CD200R1. A selected expression profile corresponding to a single-cell hierarchy topped by stem cells assembled 114 genes with decreased expression in MRUCD200/CD200R1 down to MRUnot CD200/CD200R1 and further down toward CD200+CD200R1- and CD200R1+CD200- cells. Thirty-two of these genes were integrated into integrin-based and muscle-based complexes, as well as into a connecting branch. Of the listed genes, ~40% were shared by a previously published database of upregulated genes in mammary/breast stem cells (Lim et al., 2010). Most of these common genes encoded proteins that are part of the complexes and may serve as a core of genes involved in mammary stemness. Taken together, high CD200/CD200R1 expression in mammary epithelial cells enables the elucidation of stem cells and progenitors within the MRU population. The complement system may assume a non-inflammatory role and support mammary epithelial cell regeneration.

W-1078

TISSUE ARCHITECTURE COORDINATES DIFFERENTIATION AND APICAL EMERGENCE IN THE DROSOPHILA INTESTINAL EPITHELIUM

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Epithelial tissues interface with the outside world through a specialized luminal surface which is formed collectively by the apical surfaces of differentiated epithelial cells. Although epithelia undergo constant turnover, their progenitor cells are often restricted to the tissue's basal region. To integrate properly into the tissue, new cells must thus coordinate epithelial differentiation with physical emergence into the luminal, apical surface. However, the mechanisms that achieve this coordination are poorly understood. To investigate these mechanisms, we use the intestinal epithelium of Drosophila. In this tissue, basally localized stem cells give rise to committed, but still undifferentiated, daughters called enteroblasts. Enteroblasts then undergo apical emergence and terminally differentiate into mature epithelial enterocytes. Examining these three cell types by correlative light electron microscopy, we find that enterocytes and enteroblasts, but not stem cells, form septate junctions, a structural feature of invertebrate epithelia that is analogous to vertebrate tight junctions and demarcates the apical surface. This observation suggests that the formation of septate junctions during epithelial differentiation may be a crucial control point. Indeed, genetic interference of septate junction formation in enteroblasts both prevents apical emergence and blocks full enterocyte differentiation. In this case, enteroblasts arrest in an aberrant, 'Frankenblast' state in which they exhibit hybrid characteristics of both enteroblasts and enterocytes. Our results indicate that epithelial differentiation and apical emergence are coupled by the formation of septate junctions in committed progenitors. In this manner, epithelial tissue architecture ensures that new cells integrate seamlessly into the epithelium. This mechanism may shed light on how loss of tissue architecture deregulates cell differentiation in epithelial cancers.

Funding Source: Stanford Bio-X Fellowship, EMBO Short-Term Fellowship

W-1080

EFFICIENT IN VIVO GENE EDITING USING RIBONUCLEOPROTEINS IN SKIN STEM CELLS OF RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA MOUSE MODEL

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The prokaryotic CRISPR/Cas9 system has recently emerged as a powerful tool for genome editing in mammalian cells with the potential to bring curative therapies to patients with genetic diseases. However, efficient in vivo delivery of this genome editing machinery and indeed the very feasibility of using these techniques in vivo remain challenging for most tissue types. Here, we show that nonreplicable Cas9/sgRNA ribonucleoproteins can be used to correct genetic defects in skin stem cells of postnatal recessive dystrophic epidermolysis bullosa (RDEB) mice. We developed a novel method to locally deliver Cas9/sgRNA ribonucleoproteins into the skin of postnatal mice. This results in rapid gene editing in epidermal stem cells. Using this method, we show that Cas9/sgRNA ribonucleoproteins efficiently excise exon80, which covers the point mutation in our novel RDEB mouse model, and thus restores the correct localization of the collagen VII protein in vivo. The skin blistering phenotype is also significantly ameliorated after treatment. This study provides a novel in vivo gene correction strategy using ribonucleoproteins as curative treatment for genetic diseases in skin and potentially in other somatic tissues.

Funding Source: Beijing Municipal Science & Technology Commision

W-1082

GENERATION OF KERATINOCYTES FROM INDUCED PLURIPOTENT STEM CELLS DERIVED FROM A KINDLER SYNDROME PATIENT

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Kindler syndrome is an autosomal recessive disorder caused by the mutations in the kindlin-1 gene, which is known to bind to integrins and regulate integrin activation at cell adhesions. Kindler syndrome is characterized by skin blistering, erosion and photosensitivity. Radical treatments, such as regenerative therapies using stem cells are strongly desired because of its difficulties of complete cure. Therefore, we decided to establish induced pluripotent stem cells (iPSCs) from human adipose tissue-derived stem cells (hADSCs) or keratinocytes isolated from patient with Kindler syndrome, and differentiate them into keratinocytes in order to discover the pathogenic mechanism. In this study, we have established patient-specific, transgene-free iPSCs through electroporation of episomal vectors and growth under 5% O_2 or 20% O_2 condition. Consistent with previous report, 5% O_2 significantly increased the iPSCs-like colony formation. The resulting iPSC lines were verified by the expression of pluripotent stem cell markers through immunofluorescent staining, quantitative PCR analysis, and flow cytometry analysis. Pluripotency of the iPSC lines were also confirmed by differentiation capacity into three germ layers. Then, the patient-specific iPSCs were differentiated into keratinocyte lineage through sequential applications of retinoic acid and bone-morphogenetic protein-4 and growth on collagen IV-coated plates. Keratinocytes differentiated from iPSCs displayed similar expression profiles with normal epidermal keratinocytes. We also found that iPSCs derived from patient's keratinocytes possessed a more pronounced ability to differente into keratinocyte lineage than those from patient's hADSCs. This study is expected to be a first step in the investigation of the underlying mechanism and a novel therapeutic development of Kindler syndrome.

W-1084

ORAL ADMINISTRATION OF CONDITIONED MEDIUM OBTAINED FROM AMNION-DERIVED MESENCHYMAL STEM CELL CULTURE PREVENTS ESOPHAGEAL STRICTURE AFTER ENDOSCOPIC SUBMUCOSAL DISSECTION IN PIGS

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Endoscopic submucosal dissection (ESD) for gastrointestinal neoplasms has been widely accepted in past decades; however, ESD for esophageal cancer often causes postoperative stricture when more than three-quarters of the circumference of the esophagus is dissected. To prevent stricture after ESD, balloon dilation, local injection or oral administration of steroid are generally performed. Although these methods are effective, complications such as perforation, mediastinum abscess and steroid-induced side effects are of concern. Mesenchymal stem cells (MSCs)

are a valuable cell source in regenerative medicine, and conditioned medium (CM) obtained from MSCs reportedly inhibits inflammation. In this study, we evaluated whether CM obtained from human amnion-derived MSC culture could prevent esophageal stricture after ESD. We resected a semi-circumference of pig esophagus by ESD. We prepared CM gel by mixing with 5% carboxymethyl cellulose, and endoscopically applied it onto the wound bed immediately after ESD, and on day 8 and 15 (weekly CM group), or orally administered from day 1 through day 4 (daily CM group). We also injected triamcinolone acetonide into the remaining submucosa immediately after ESD (steroid group). We euthanized the pigs on day 8 or day 22 to measure the stricture rate and perform histological analysis. Stricture rate in weekly and daily CM groups and steroid groups were significantly lower than in the control group on day 22 (56.3±7.1%, 52.7±19.3% and 49.3±4.2% vs 80.0±2.0%, respectively). Moreover, CM significantly attenuated the number of activated myofibroblasts (26.8±8.6, 21.5±4.9 and 20.6±2.3 vs 68.3±5.7 cells/μm2) and fiber thickness (833±26, 987±145 and 944±251 vs 1,609±418 μm) on day 22. Oral administration of CM also significantly decreased the infiltration of neutrophils and macrophages compared with the control group on day 8 (31.7±5.9 vs 68.1±14.2 and 13.2±1.7 vs 33.9±2.8 cells/HPF, respectively). In conclusion, CM gel prevents esophageal stricture formation by suppressing myofibroblast activation and fibrosis following the infiltration of neutrophils and macrophages. Oral administration of CM gel would be a promising treatment for the prevention of post-ESD stricture.

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W-1086

QUIESCENT ZEB1+ CELLS ARE IMPORTANT FOR MOUSE MAMMARY REGENERATION & TUMORIGENESIS

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Quiescent stem cells exist in many to avoid genomic insults and prevent exhaustion caused by excessive proliferation. Our previous work identified the multipotent mammary stem cells (MaSCs) by Protein C Receptor (Procr) expression. In this study, we discovered the heterogeneity of Procr+ MaSCs and found that half of the Procr+ MaSC population expressed Zeb1. To study Zeb1+ cells and the role of Zeb1 in development, we generate Zeb1-CreERT2 and Zeb1-flox mouse models. Lineage tracing of Zeb1+ cell indicated that they are rare, quiescent and long-lived in homeostasis. Knockdown of Zeb1 did not affect MaSC colony formation capacity, while Zeb1 conditional knockout did not affect mammary development. However, Knockdown of Zeb1 led to impaired mammary regeneration and inhibition of MMTV-Wnt1 mammary tumor formation, suggesting that this novel quiescent population, marked by Zeb1 expression, is important for regeneration and tumorigenesis.

STEM CELL NICHES

W-1088

ACTIVATION OF THE MOUSE RESIDENT SKELETAL STEM CELL FOR ARTICULAR CARTILAGE REPAIR

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Osteoarthritis (OA) afflicts 15% of the adult population, with a lifetime risk of 40%. In OA, the articular cartilage made up of hyaline has a low propensity for regeneration. Microfracture (MF) is believed to encourage stem cells from the bone marrow to form new fibrocartilage. We have identified the mouse Skeletal Stem Cell (mSSC) with its entire lineage and have identified a subset of CD49f positive mSSC activated after bone injury.Our aim is to examine the effect of MF on articular cartilage in activating the native mSCC and Bone Cartilage Stromal Progenitor (BCSP) population and direct their fate towards chondrogenesis. We performed MF on the articular surface of the left distal femur of skeletally mature, 9week old male mice. At each time-point (1,2,4weeks post-op) we euthanized the mice and assessed the populations of SCC and BCSP in MF and control right femurs by Fluorescence Assisted Cell Sorting (FACS). We assessed clonality by using our Rainbow mouse model. Histological composition was assessed using Movat's Pentachrome Stain. Mice were operated in groups of 10 for each time-point. Statistically significant increases in the mSCC and BCSP populations followed MF. The maximum effect of MF on mSCC and BCSP populations was seen at week 2. We also show preliminary data, indicating the MF activated mSSC can be directed towards cartilage differentiation through the action of recombinant factors that we administer exogenously. Our MF model that we will develop is an easily translatable, clinically-relevant therapy.

W-1090

EXTRACELLULAR AND INTRACELLULAR MMP12: DISTINCT FUNCTIONS IN THE POSTNATAL DEVELOPMENT OF THE SVZ NEURAL STEM CELL NICHE

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The subventricular zone (SVZ) houses the largest neural stem cell (NSC) niche in the mammalian brain. In the adult SVZ, molecular cues from specialized niche support cells and extracellular matrix (ECM) tightly regulate NSC function. Multiciliated ependymal cells arise postnatally from NSCs and adopt a distinct planar cell polarity to coordinate ciliary movement. The apical surfaces of NSCs become surrounded by pinwheels of ependymal cells along the ventricle surface, with distinctive hubs of ECM at NSC-ependymal cell interfaces. This dramatic cellular and ECM remodeling led us to hypothesize that matrix metalloproteinases (MMPs), a family of endopeptidases that regulate cellular and ECM remodeling in many tissues, may be important in forming the postnatal SVZ niche. We found that, among all 24 mouse MMPs, MMP12 is particularly upregulated during ependymal cell maturation. Surprisingly, we discovered that in an MMP12 mutant mouse line, a truncated, intracellular isoform of MMP12 (icMMP12) remains expressed in ependymal cells. Extracellular MMP12 has recently been found to translocate into cells and function as intracellular proteinase and transcription factor, but an endogenous icMMP12 has not been previously reported. To tease out the functions of extracellular and intracellular MMP12 in the developing SVZ, we took advantage of the MMP12 mutant mice as an extracellular MMP12 knockout model, and combined it with in vivo MMP12 knockdown, icMMP12 overexpression, as well as cell permeable and impermeable MMP12 inhibitors. We found that extracellular MMP12 regulates the development of SVZ niche structures, including pinwheel organization, the formation of ECM hubs, and ependymal cell planar cell polarity, while ciliogenesis in ependymal cells is unaffected by extracellular MMP12. In contrast, icMMP12 has a unique function in regulating ependymal ciliogenesis, which may be related to actin cytoskeleton organization. Finally, we found that icMMP12 regulates SVZ niche output by suppressing NSC proliferation. Together, these findings describe a novel icMMP12 isoform in ependymal cells, and reveal the unique roles of extracellular and intracellular MMP12 in the development of the SVZ NSC niche.

W-1092

ROLE OF PDGF-BB IN HUMAN PDL STEM CELLS PROLIFERATION, MATRIX SYNTHESIS AND MINERALIZATION

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Platelet-derived growth factor-BB (PDGF-BB) is one of the most abundant growth factors in human platelets and has been shown to significantly participate in regeneration after tissue injury. There is a population of mesenchymal stem cells in human periodontal ligament (PDL) which can contribute to tissue regeneration under appropriate conditions. PDL cells were isolated and characterized using stem cell and differentiation markers via immunofluoresce and flow cytometry and then cultured in vitro and treated with different concentrations of PDGF-BB. The effect of PDGF-BB on cell proliferation, stem cell and differentiation markers expression, soluble collagen production, lysyl oxidase (LOX) activity, alkaline phosphatase (ALP) activity and calcium nodules formation was assessed. PDGF-BB stimulated the proliferation of cells with the maximum effect at 50 ng/mL. The growth factor increased the expression of stem cell markers and SPARC; Col1a2 expression was decreased, whereas the expression of Col3a1 and BSPII remain unchanged. Soluble collagen production, ALP activity and calcium nodules formation were also significantly decreased by PDGF-BB; LOX activity was significantly increased. PDGF-BB is a powerful promoter of cell proliferation and increases the expression of stem cell markers; inhibits collagen production and mineralization but accelerates the maturation of collagen chains through increased LOX activity and SPARC expression.

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W-1094

ROLE OF CCL4 AND CCR5 DURING OSTEOCLASTOGENESIS OF MURINE BONE MARROW-DERIVED MONOCYTES

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Chemokine CCL4 (MIP-1β) is released from osteoblast cells to restore the homeostasis of hematopoietic stem cells during the activation of bone marrow. In this study, the function of CCL4 during osteoclastogenesis

was investigated. CCL4 promoted the migration and viability of pre-osteoclast cells. However, CCL4 had no direct effect on the receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclastogenesis in mouse pre-osteoclast cells. During osteoclastogenesis, the expression of CCR5, the CCL4 receptor, was rapidly reduced by RANKL treatment. CCR5 down-regulation by RANKL was mediated by MEK and JNK in pre-osteoclast cells and played a role in osteoclastogenesis. These results suggest that the chemoattractant effect and viability of CCL4 is involved in recruiting pre-osteoclasts but is diminished later its effect on osteoclastogenesis by the reduction of CCR5 when RANKL is prevalent.

W-1096

BMP4 AND FGF8 AFFECT DTPCS PROLIFERATION AND DIFFERENTIATION

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The aim of this research is to isolate primary mesenchymal stem cell cultures from deciduous tooth pulp and to characterize them for expression of specific mesenchymal markers using immunofluorescence and flow-cytometry. The cells were characterized for expression of stem-cell markers (Nestin, Vimentin, CD146, CD44, CD49f) and markers associated with dental tissues (ALP, COL1A2, COL3A2, DSPP) using flow-cytometry and immunofluorescence. Primary mesenchymal stem cell cultures from deciduous tooth pulp were isolated from routinely extracted, healthy deciduous teeth. For this research cells between 4-th and 6-th passages were used. The results showed that the mesenchymal stem cell cultures are heterogeneous. We proved that there are cells expressing characteristic reparative dentinogenesis markers COL1A2, COL3A1, DSPP, ALP as well as stem cell markers nestin, vimentin, CD44, CD49f, CD146. After revealing the phenotype of DTPSC we treated the cell cultures with 10 ng/mL FGF8 or BMP4 for 48 h. Cell proliferation, collagen production and expression of differentiation markers COL1A2, DSPP and ALP were assessed. FGF8 was found to significantly increase cell proliferation. Both growth factors increased expression of differentiation markers COL1A2, DSPP and ALP. Soluble collagen secretion in the supernatant was also significantly increased by both BMP4 and FGF8. Revealing the phenotype of the mesenchymal stem cells from deciduous teeth pulp will give us invaluable information about the potential of these cells and will help us to better understand the regenerative potential of tooth pulp and the processes of dentine production and reparation. Interaction between FGF8 and BMP4 sets the beginning of differentiation of both odontoblasts and ameloblasts. Revealing the intimate interaction mechanism between FGF8 and BMP4 in DTPSC may lead to developing new dental regenerative techniques.

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W-1098

REVEALING THE HIERARCHICAL LINEAGE RELATIONSHIP BETWEEN PRIMITIVE AND DEFINITIVE NEURAL STEM CELLS IN THE ADULT MOUSE FOREBRAIN

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Adult neural stem cells (NSC) reside in the periventricular region along the entire neuraxis. Two distinct populations of NSCs persist into adulthood: a rare, Oct4 expressing primitive NSC (pNSC) and a more abundant, GFAP expressing definitive NSC (dNSC). Previous studies using in vitro passaging and in vivo cell transplantation have supported the lineage relationship between the distinct NSC populations, indicating that pNSCs lie upstream of the dNSCs. Herein we have performed in vivo experiments that provide direct evidence to the lineage hierarchy. We have built on the finding that the specific ablation of dNSCs invariably results in their return over time. Using various transgenic mouse models we first confirmed that dNSC and pNSC have distinct, non-overlapping marker expression (Oct4 in pNSCs, GFAP in dNSCs). In GFAPCreERT2;ROSAyfpfl/fl;GFAPtk mice, tamoxifen (tam) labels a cohort of GFAP+ dNSCs. The subsequent, specific ablation of GFAP expressing dNSCs using GCV leads to an initial, complete loss of dNSCs however, the dNSC population returns over time (as previously reported). In all cases, the repopulation is from a GFAP negative cell, supporting the hypothesis that the GFAP negative pNSCs are activated to repopulate the dNSCs. In a separate set of experiments, GFAPtk;ROSAyfpfl/fl mice injected with a Cre-recombinase expressing retrovirus (RV) during dNSC ablation (i.e. during GCV infusion) lead to a small percentage of YFP expressing dNSCs in addition to YFP+ pNSCs. Hence, GFAP-negative, pNSCs were proliferating during dNSC repopulation. Finally, we took advantage of the unique marker expression of Oct4 in pNSCs to selectively label pNSCs in dNSC depleted mice (tam fed Oct4CreERT2;ROSAyfpfl/fl;GFAPtk receiving GCV). As predicted, some dNSCs that returned over time expressed YFP, revealing a direct contribution from pre-labeled Oct4 expressing pNSCs. Together, these findings confirm the NSC lineage relationship in vivo, whereby the pNSC lies upstream of the dNSC in the adult forebrain.

EYE AND RETINA

W-1100

CONTINUOUS BLOCKING OF THE DIFFERENTIATION OF RETINAL PROGENITOR INTO NON-CONE FATES PERMITS THE ISOLATION OF LARGE CONE PHOTORECEPTOR SPECIFIC RETINAL PROGENITOR CLONES

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Adult retinal stem cells (RSCs) are rare pigmented cells in the ciliary epithelium of the retinal periphery of many mammals, capable of giving rise to all types retinal neurons, including cone and rod photoreceptors. Here we report the induction and characterization of large purified populations of cone photoreceptors from mouse RSC progeny by way of the induction of cone specific retinal progenitors. Following 28 days of COCO (a TGFβ, Wnt and BMP antagonist) treatment added to pan-retinal (1% fetal bovine serum + fibroblast growth factor + heparin) conditions during the differentiation of RSC progeny, 56% of RSC progeny were positive for cone arrestin and 46% were positive for S-opsin (mature cone markers). In contrast, in pan-retinal differentiation conditions alone, RSC progeny produced clones with 0 - 1% cones. A similar enrichment of cone photoreceptor differentiation was seen with COCO treatment of embryonic neural retina clones. The COCO induced cone arrestin positive cells did not express the rod marker rhodopsin or the retinal pigmented epithelial cell (RPE) marker (RPE65), suggesting COCO is not inducing RSC progeny to ectopically express photoreceptor or RPE genes non-specifically. Next, we sorted undifferentiated clonal RSC progeny into single non-pigmented (NP) or pigmented (P) progenitors, plated them at a single-cell-per-well and differentiated the clones in COCO or pan-retinal control conditions for 45 days. We found that only clones from NP progenitors in COCO were cone arrestin positive (nearly 100% of the clones consisted of cone only clones, including clones of several hundred cells), suggesting that COCO may act in a cell specific inductive manner. Moreover, RNA sequencing analysis revealed a very close gene expression similarity between the endogenous cones and the cones produced from retinal progenitors in COCO. Most important, COCO must be present throughout the entire differentiation period to allow differentiation of large numbers of cone photoreceptors. These data suggest that the NP neural retinal progeny of RSCs can differentiate into cone photoreceptor-specific progenitors by default if their differentiation into non-cone fates is inhibited.

Funding Source: CIHR, CIHR Biotherapeutic, Medicine by Design, Foundation Fighting Blindness Canada, Krembil Foundation, OIRM

W-1102

GENERATION OF MESC-DERIVED CONE PHOTORECEPTOR PRECURSORS

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Cell-based strategies by means of photoreceptor transplantation have been shown to be a feasible therapeutic approach for retinal degeneration diseases. Indeed, after transplantation of primary rod/cone – photoreceptor precursors, some visual restoration has been observed in different mouse models of retinal degeneration. Nonetheless, in the human system, primary photoreceptor precursors are not the ideal cell source for such treatment approach, since transplantable photoreceptor precursors can only be obtained at embryonic stages of development, which is ethically and legally concerning. Therefore, derivation of photoreceptor precursors from pluripotent stem cells (PSC) is being currently developed as an alternative cell source. Several studies have already demonstrated the ability of mouse/human embryonic/induced pluripotent stem cells (m/h E/iPSC) to differentiate into 3D retinal tissue, so called retina organoids, in a self-organised manner. Rod photoreceptor precursors have been isolated from retinal organoids via fluorescent/magnetic – activated cell sorting (FACS/MACS respectively) and have been shown to survive and mature after transplantation into the sub-retinal space of retinal degeneration mouse models. In contrast to the nocturnal mouse, human vision highly depends on cone photoreceptors that are active in daylight conditions. As rods represent the vast majority of photoreceptors in the mouse retina and PSC-derived retina organoids, factors required for cone photoreceptor cell fate acquisition are not well studied. This lack of knowledge leads to a delay in the development of cell therapies for cone degeneration diseases compared to those for rod degeneration. Therefore, we are currently developing and optimising protocols and tools that allow us to increase the amount of cone photoreceptor precursors and detect them in retinal organoids for further development of cell therapies tailored for cone degeneration diseases.

W-1104

HUMAN RETINAL GANGLION PROGENITOR CELL INTEGRATES INTO RETINAS IN A MURINE MODEL

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Retinal ganglion cells (RGC) are retinal neuronal cells that form the inner layer of retina and the axons of these cells form the optic nerve. Loss of RGC is a significant cause for vision loss in glaucoma and other optic neuropathies. Optic nerve crush (ONC) is an acute optic nerve damage animal model which is used in many studies of optic neuropathy. We investigated whether human RGC progenitor cells could be integrated into the appropriate retinal layer post ONC in a murine model. Unilateral ONC was performed surgically on 6-8 weeks old C57BL/6 mice. After mice were anesthetized, a conjunctival incision was made on the temporal side and optic nerve was exposed following blunt dissection. Retinal progenitor cells were provided by Astellas Institute for Regenerative Medicine. Immediately after ONC, 1x105 RGC progenitor cells in 1 µl saline were injected intravitreally using a 33-gauge needle. Vehicle was injected as control. Mice were treated with cyclosporine in the drinking water to prevent rejection of cell transplant from 2 days prior to transplant through the duration of the experiments. At the end of each study (2-6 weeks) retinas were dissected and wholemount retinas and frozen vertical sections were analyzed for incorporation of the cells into the retina using immunofluorescent staining with RBPMS (RGC marker) and antibodies to reveal cells of human origin. Staining of wholemount retinas showed the presence of human cells within the retina at 2 weeks (n=23 mice), 4 weeks (n=10 mice) and 6 weeks (n=10 mice) post cell transplant. Confocal microscopy identified colocalization of human cells with RBPMS positive cells, indicating that some of the RGC progenitor cells are in the RGC lineage. Staining of retinal vertical cross sections showed cells of human origin at 4 and 6 weeks in the RGC layer as well as in vitreous and they co-expressed RGC marker RBPMS. RGC progenitor cells integrate into retina and survive at least 6 weeks post ONC. Some of the cells gain the RGC transcription factor RBPMS, suggesting differentiation towards the RGC lineage. Additional work is needed to examine the efficacy of cell transplantation after the establishment of ONC and examine the long-term survival and axonal regrowth of RGC progenitor cells in the retina.

W-1106

TRANSCRIPTOMIC PROFILING OF EPITHELIAL TO MESENCHYMAL TRANSITION IN STEM CELL-DERIVED RETINAL PIGMENT EPITHELIUM

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RPE stress and injury often lead to RPE dedifferentiation and epithelial to mesenchymal transition (EMT), whose characteristics include alteration of the RPE's normal cobblestone morphology to a more spindle-shaped fibroblast morphology. RPE dysfunction and EMT have been implicated in a number of retinal diseases. In order to better understand the molecular mechanisms of EMT in the human RPE, and determine its similarities and differences to EMT processes associated with malignant transformation in other cell types, we used RNA-seq to determine the transcriptome of human stem cell-derived RPE cells induced to undergo EMT. Human iPS-RPE monolayers were cultured using our previously published methods (Maruotti et al, 2013 and 2015). For inducing EMT, RPE monolayers were enzymatically dissociated and re-plated for 3, 12 and 48 hrs in duplicates. Un-dissociated RPE monolayers were considered as controls. First and second strand synthesis was performed from total RNA using anchored oligo-dT and DNA Polymerase I respectively. RNA-seq libraries were constructed using the Nextera XT DNA Library Preparation Kit and sequenced on an Illumina HiSeq 2500 with 50 bp paired-end reads. Reads were aligned to NCBI build 37.2 using Tophat (v2.1.0). Cuffquant and Cuffnorm (Cufflinks v2.2.1) were used to quantify expression levels and calculate normalized FPKM values. Pathway analysis was performed using Gene Set Enrichment Analysis (GSEA) and validated by qRT-PCR. The key mesenchymal transcription factors SNAI1, ZEB1, and TWIST1 were up-regulated, whereas the RPE-specific markers MITF, BEST1, RPE65, RLBP1, and LRAT were down-regulated after the dissociation of monolayers. GSEA uncovered a number of key signaling pathways, including ECM-receptor, focal adhesion, TGF-β, NOTCH, and WNT/TCF. Our data provide a novel insight into both positively/negatively correlated transcription factors and miRNA-regulated target genes whose expression is altered by the induction of EMT in human RPE cells. Temporal transcriptome analysis of

RPE EMT enables the identification of early and late intrinsic molecular pathways and their possible link to RPE degeneration. Moreover, defining the transcriptional networks involved in human RPE cells undergoing EMT may provide novel therapeutic targets for treatment of retinal diseases.

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NEURAL DEVELOPMENT AND REGENERATION

W-1108

POST-TRANSCRIPTIONAL REGULATION OF MOUSE NEUROGENESIS BY PUMILIO PROTEINS

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Mammalian neurogenesis occurs during fetal development and persists throughout adult life. Despite extensive studies of transcription, epigenetics, and cell-cell signaling in regulating this process, little is known about post-transcriptional control. Here we report the neurogenic function of two post-transcriptional regulators in mice, Pumilio (Pum) 1 and 2, of the evolutionarily conserved PUF RNA binding protein family. PUF proteins are known to mediate post-transcriptional regulation in stem cells and development in lower organisms, but their function in mammalian neurogenesis remains unknown. We show that Nestin-cre driven neural specific conditional double knockout of Pum1; Pum2 led to severely reduced number of neural stem cells (NSCs) in the dentate gyrus (DG) after birth, drastically increased perinatal apoptosis, accumulation of Tbr2+ neuronal progenitors but fewer DCX+ immature neurons, as well as largely impaired learning and memory. Consistently, in neurosphere assays, the mutant DG gave rise to fewer NSCs which displayed defects in proliferation, survival and differentiation in vitro. All these defects indicate a major role of Pum1 and Pum2 in hippocampal neurogenesis and function. To investigate Pum-mediated mechanism, we employed Pum1/2 crosslinking immunoprecipitation (iCLIP) assay, which detected Pum binding sites at single nucleotide resolution and identified 3,588 Pum1- and 2,763 Pum2-target mRNAs, with 1,476 as common targets that are involved in multiple pathways crucial to neurogenesis. The majority of Pum targets showed upregulation at the protein level but remained unchanged at the transcript level upon depleting Pum1 or Pum2 or both. Hence, Pum1 and Pum2 achieve their neurogenic function by post-transcriptionally regulating their RNA targets. Lastly we discovered an RNA-dependent interaction between Fmrp and Pum. Pum1, Pum2, and Fmrp are targets of each other. While depletion of Fmrp did not affect Pum binding, depletion of Pum2 or both Pum proteins significantly diminished Fmrp binding, supporting the interaction between Fmrp and Pum in regulating neurogenesis, including the role Pum2 plays in facilitating Fmrp function. In addition, the difference between Pum1 and Pum2 in interacting with Fmrp might indicate differential roles of Pum1 and Pum2 in neurogenesis.

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W-1110

LONG TERM STABILITY OF L-MYC EXPRESSING NSCS: DEVELOPMENT OF COMPUTATIONAL PREDICTION MODELS FOR PERSONALIZED MEDICINE

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Several preclinical studies indicate that neural stem cells (NSCs) can limit or reverse central nervous system (CNS) damage through cell replacement, regeneration, or delivery of therapeutic agents to sites of degeneration or tumor. Allogeneic NSC lines are in growing demand due to the limitations of autologous stem cells including time for modification, costs and availability to patients. We generated and characterized a new human NSC line, immortalized by transduction with a single gene, transformation deficient L-myc (LM-NSC008). At present study, we demonstrated long-term fate, stability and luck of tumorigenicity of LM-NSC008 cells in vitro and in non tumor-bearing naïve mouse brain (up to 9 month). LM-NSC008s displays self-renewal and stable L-myc expression up to passage 50 in vitro and were easily expanded to a large cell banks using Quantum fiber bioreactor. Cortically-injected LM-NSC008 cells

migrate long distances across hemispheres and generate specialized cell types through differentiation in vivo. In mouse xenograft models of brain tumors and injury, intranasally-administered cells showed tumor homing as visualized in thick cleared brain sections. These data approaches for NSC administration and therapeutic targeting will support further use of LM-NSC008 cells in brain tumor and injury models to explore novel and noninvasive routes of stem cell delivery to the brain. The inability to visualize, quantitatively analyze, and predict exogenous stem cell migration is a barrier to successful translation of stem cell therapies to the clinic. The developed computational model of NSC migration in the brain which provides a way to predict the numbers of NSCs that will reach a tumor depending on the injected dose, route of delivery and location of the target site within the brain. This method could also be used in areas of brain injury and regenerative medicine. This computational tool may be used to identify brain tumor patients that are good candidates for NSC therapy depending on tumor/injury location, or the best NSC administration method for a patient.

Funding Source: Alex Lemonade Stand Foundation

W-1112

DERIVATION OF HUMAN EXCITATORY NEURONS WITH NMDAR-MEDIATED SYNAPTIC TRANSMISSION FOR DISEASE MODELING

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Neuronal populations derived by direct reprogramming of pluripotent stem cells retain an immature phenotype, and modeling mature synaptic transmission remains challenging. This in turn impedes disease modeling and drug discovery. Here, we combined forced expression of NGN2 with small molecule patterning via inhibition of SMAD signaling to induce neuronal differentiation in hPSCs. We find that this approach generates homogenously patterned excitatory cortical neurons, which we named human patterned induced neurons (hpiNs). Using single-cell RNA sequencing, along population RNA sequencing and electrophysiological recordings, we identified CAMK2A-expressing highly differentiated and synaptically active hpiNs that expressed AMPA and NMDA receptor subunits. This underscores the utility of this approach for modeling diseases associated with glutamate receptor dysfunction, including schizophrenia, epilepsy and autism. Finally, we describe a pipeline for defining statistically sound and reproducible cellular, molecular, and physiological phenotypes associated with disease.

W-1114

GFAP-NEGATIVE NSCS IN THE PERI-NATAL MOUSE SEZ DIRECTLY MAKE ASTROCYTES VIA A GFAP-NEGATIVE PROGENITOR

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We define two distinct types of neural stem cells (NSCs) in the developing and adult mouse brain that generate the diversity of neural progenitor cells (NPCs) that build the brain. Primitive (p)NSCs do not express GFAP, and form clonogenic neurospheres when grown in LIF. PNSCs arise ~E5.5 and persist into the adult brain, where we have shown that they are very rare and quiescent, express Oct4, and give rise to GFAP+ definitive (d)NSCs that form neurospheres in EGF and FGF2. We used the neurosphere assay in vitro to enrich for progenitor cells downstream of either NSC type to characterize the functional differences between these two NSC populations. Here we show both NSC types derived from the E17.5 mouse brain give rise to NPCs that are bipotent in the neuronal and glial lineages, though pNSCs give rise to many NPCs specified to only make neurons. Interestingly, progenitor cells from either NSC that gave rise to both lineages generated significantly more progeny than those committed to either glial or neuronal fates, suggesting that multipotent progenitors proliferate more than unipotent progenitors. As pNSCs can give rise to dNSCs as well as neurons and glia, we asked whether pNSCs can directly produce neurons and glia without a dNSC intermediate step. To test this, we grew clonal spheres from early postnatal mice with herpes simplex virus thymidine kinase expression driven by the GFAP promoter, which causes GFAP-expressing cells (ie. definitive NSCs) to be killed upon division following administration of ganciclovir. There is a significant reduction in the number of neurospheres that form in EGF and FGF2 from definitive NSCs from the subependymal zone (SEZ) of early post-natal mice with this manipulation, as expected. Preliminary results show pNSCs from these mice could still form GFAP+ astrocytes, suggesting that downstream of pNSCs, astrocytes are made via a progenitor that is GFAP- while it is proliferative. By combining the retroviral lineage tracing and GFAP-TK data, we have constructed a novel hierarchy for progenitor cells downstream primitive and definitive neural stem cells. We will continue to explore

this heterogeneity of neural precursors using single cell RNA-sequencing.

W-1116

DERIVING SENSORY SPINAL INTERNEURONS FROM HUMAN PLURIPOTENT STEM CELLS

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Spinal sensory interneurons (INs) integrate and relay somatosensory information, such as the experience of touch or pain. The loss of somatosensation after injury or disease can be debilitating both physically and emotionally. One means of restoring sensory function is to repopulate diseased or damaged areas of the nervous system with stem-cell derived sensory INs. We are working towards this goal by developing directed differentiation protocols based on the mechanisms that specify sensory INs during spinal cord development. Sensory INs arise as a result of signaling from the Bone Morphogenetic Proteins (BMPs) family of growth factors. BMPs pattern the dorsal spinal cord, including the progressive production of roof plate (RP) cells at the dorsal midline, and the dI1 and dI3 populations of sensory INs, which mediate proprioception and mechanosensation respectively. As a first step, we have determined the culture conditions in which BMPs can induce dorsal spinal IN fates from mouse embryonic stem cells (mESCs). We have shown that BMP4 and BMP6 specifically direct mESCs toward different dorsal identities: BMP4 is most effective at directing mESCs into dI1s and dI3s, whereas BMP6 allows for efficient differentiation into RP cells. As a second step, we are now assessing whether these BMPs can similarly direct human stem cells towards dorsal spinal fates. The successful implementation of these protocols will permit us to work towards the ultimate goal of restoring sensory function using patient specific INs generated in vitro.

Funding Source: California Institute for Regenerative Medicine (CIRM)

W-1118

PATIENT-SPECIFIC NEURAL STEM CELL THERAPY REVERSES AN ALZHEIMER'S DEMENTIA SYNDROME IN OLDER PET DOGS

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Dementia currently affects 32 million individuals worldwide, with projections of 130 million by the year 2050. Due to limited progress in medical management dementia remains an incurable and fatal disorder. The underlying clinicopathologic issue in early dementia is mass neuronal loss in the hippocampus. Repopulation by exogenous neural precursors is therefore a promising therapeutic strategy but has yet to reach clinical trial. One of the major challenges has been poor translational fidelity between rodents and humans. We therefore have focused on Canine Cognitive Dysfunction (CCD), a neurodegenerative disorder in older pet dogs with many parallels to human Alzheimer's dementia. Dogs with CCD display amnesia, spatial disorientation and agitation, and express neurodegeneration alongside Alzheimer pathology. To date, we have produced >50 genetically non-modified skin-derived neural precursor (SKN) lines from adult canine skin. These are highly homogenous in culture (CD133+Nestin+P75+ >97.9%; CV < 1.8%), rate-limited by virtue of low number of maximal cell doublings, and differentiate almost exclusively into neurons, endogenously upregulating neuronal specification genes Ascl1, Brn2, Mytl1 and NeuroD ($p=0.04$). We show that canine SKN transplantation into the aged rodent hippocampus is safe and leads to widespread neuronal engraftment. Donor cells become electrophysiologically active, and integrate synaptically into host neuronal circuitry. Moreover, we observe rescue of hippocampal-dependent place recognition memory deficits, with normalized exploration ratio restored from categorically impaired (0.57), back to levels equivalent with young rats (0.71; $n=9$; $p=0.02$; $d=1.1$). Accordingly, we are now assessing the safety and efficacy of our SKN therapy in a world-first therapeutic trial to treat dementia in a higher-order animal model. We can report that 18-months following MRI-guided intra-hippocampal injection of autogenic SKNs, two consecutive patients demonstrate stable and clinically meaningful improvement in CCD signs, such that they

are functionally cured. These results are paralleled by dramatic improvements on objective spatial memory testing. These exciting early trial results indicate that SKN therapy can in-principle reverse a naturalistic dementia-like syndrome.

W-1120

GENERATION OF A NERVE ORGANOID WITH HUMAN STEM CELL-DERIVED NEURONS

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During development, axons spontaneously assemble into a bundled structure to form nerves and tracts in the nervous system as they extend within a spatially constrained path. However, understanding of the axonal fascicle has been hampered by lack of an in vitro model system. Here, we report the generation of a nerve organoid bearing a robust fascicle of axons extended from human stem cell-derived motor neurons within our custom-designed microdevice. The device is equipped with a narrow channel providing a microenvironment that mimics the in vivo axon growth path and facilitates the growing axons to spontaneously assemble into a unidirectional fascicle. We characterized the fascicle and found that it was specifically made with axons, electrically active, and elastic. Our data suggests that the nerve organoid can be used as a model for evaluating degeneration of axon bundles in vitro. This nerve organoid model should allow future studies on the development of the axonal fascicle and facilitate drug screens aimed at diseases affecting axon fascicles.

W-1122

ASCL1 INDUCED NEURONAL DIFFERENTIATION USING A NOVEL TRANSCRIPTION DELIVERY VEHICLE

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During spinal cord injury (SCI), both primary and secondary injury events lead to a substantial reduction of both neuronal and glial cells. This cell death, in turn, results in significant inhibition of neuronal communication and an ensuing loss of both motor and sensory function. While there are pools of endogenous neural stem cells, a variety of local environmental factors inhibit both their differentiation and migration to the injury site. Although endogenous sources of stem cells require extra stimulation, they do have the potential to circumvent many issues commonly encountered by exogenous stem cell use. One mechanism by which endogenous stem cell differentiation is induced is via the use of either growth factors and/or use of gene manipulation via viral transduction. However, such treatments also have significant side effects. Here we propose the use of a novel mechanism of transcription factor delivery. Using a modified fusion peptide consisting of the transcription factor Achaete-scute homolog 1 (ASH1) and a modified internalization peptide sequence, we can reliably transfer the transcription factor into endogenous stem cells both in vitro and in vivo. ASH1 is a transcription factor required for neuroblast differentiation into neurons. Using a model of SCI in the rat, we delivered this fusion construct to the endogenous stem cells surrounding the injured spinal cord. Using gait analysis, immunocytochemistry and electrophysiology, we demonstrated that ASH1 treatment lead to improvements in both gait stride and electrophysiological function of the injured rat spinal cord. Furthermore, in vitro experiments demonstrated that this fusion peptide construct induced neuronal differentiation in mouse neural stem cells. These findings demonstrate that this technology has the capacity to reliably induce stem cell differentiation in the injured organism and thus potentially lead to improved treatments for related injuries such as SCI and stroke.

Funding Source: NSERC Engage grant; Saskatchewan Health Research Foundation research grant

W-1124

SOX2 AND B-CATENIN COMPETE AT BINDING SITES IN THE ATOH1 3'-ENHANCER DURING EARLY DEVELOPMENT OF MURINE INNER EAR HAIR CELLS

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Sox2 and β-catenin are important transcription factors in the regulation of proliferation and differentiation of stem cells during development and regeneration. Interactions of Sox2 and β-catenin with binding partners determine the biological role of these transcription factors and their role is level-dependent. Sox2 and β-catenin have been demonstrated to be essential for specification of the prosensory domain in the inner ear and for differentiation into hair cells during development. Atoh1 is a key regulator of hair cell differentiation and its 3'-enhancer is an important site for regulation. It can drive transdifferentiation and hair cell regeneration after injury in addition to its role in development. Sox2 and β-catenin bind at two sites in the Atoh1 enhancer. The binding sites for each transcription factor overlap, and the two factors can independently regulate Atoh1 expression. ChIP, mRNA and luciferase analysis of Sox2 and β-catenin interaction at the Atoh1 3'-enhancer show a complex competitive mechanism. High levels

of Sox2 drive proliferation over differentiation, while low levels of Sox2 upregulate Atoh1 when β-catenin levels are low. High levels of β-catenin however, are required to drive later differentiation and involve DNA-binding of β-catenin and upregulation of Atoh1 and downstream targets in the absence of Sox2 binding. The interplay between Sox2 and β-catenin is essential for Atoh1 regulation during development and might be useful in efforts to drive Atoh1 upregulation in hair cell regeneration.

W-1126

RESTORATION OF REELIN/APOER2 INTERACTION RESTORE COGNITIVE DYSFUNCTION OF DEMENTIA MODER MICE AFTER TRANSPLANTATIOB OF HUMAN IPS CELL-DERIVED NEURAL STEM/PRECURSOR CELLS

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[Purpose] Cell replacement is one of the radical treatments on regenerative medicine. We have already reported that transplantation of neural precursor cells derived from human iPS (hiPS) cells restored the spatial memory learning of human APP transgenic mouse (PDAPP) in last year's ISSCR. In this conference, we are going to show that Reelin is a key molecule in improving cognitive function. Several circumstantial evidences that Reelin is involved in the pathology of dementia have been proposed, but in either case there are few results showing direct involvement in circumstantial evidence. [Materials and methods] (Culture of hiPS cells) The hiPS cell lines, 253G1 (RIKEN, Tsukuba, Japan), were used in this study. We first developed embryoid bodies (EB). Then EB were cultured on fibronectin (FN)-coated dishes and we added retinoic acid (RA), noggin-Fc (NOG) and sonic hedgehog (SHH). (Transplantation and Morris water maze(MWM) test) We transplanted the neuronal precursors into the PDAPP mice at day 8. Neuronal precursors derived from hiPS cells (2 x 105 cells per 2μl of saline; n=21) and PBS (n=19) were stereotaxically transplanted into hippocampus. MWM test was conducted 14 days before and 15 days after the transplantation to assess the spatial memory learning of PDAPP mice. [Results] The expression of Reelin was 29.0±1.8% in wild type mice (B6, n=3), but 19.0±1.8% in dementia model mice (PDAPP, n=3). It was revealed that expression was decreased in mice with decreased cognitive function. After neural transplantation, the expression of Reelin was elevated in mice with improved cognitive function. Furthermore, the expression of ApoER2, which is a receptor of Reelin was also up-regulated in host brain, and activation(pshosphorylation) of the ApoER2 downstream signaling molecules (DAB1, Akt, GSK3beta) was also observed. [Conclusions] After transplantation, expression of Reelin and its receptor ApoER2 was remarkably enhanced in the host brain. Our results suggested that decreased expression of Reelin negatively affects cognitive function and that increased expression of Reelin may lead to cognitive function improvement.The restoration of Reelin/ApoER2 interaction may be importantly associated with the improvement of cognitive dysfunction in the dementia model mice.

W-1128

SURVIVAL AND INTEGRATION OF STEM CELL-DERIVED MOTOR NEURONS TRANSPLANTED TO THE MOUSE HYPOGLOSSAL NUCLEUS

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Lesions in the central nervous system (CNS) often lead to permanent neurological deficits due to limited regenerative capacity. It is therefore essential to develop methods to replace lost neurons in order to restore function. Transplantation of stem cell-derived neurons is an attractive option, but inefficient integration of grafted cells with host tissue constitutes an obstacle before clinical applications can be considered. In this project, we developed a mouse model for studying transplantation, survival and integration of motor neurons (MNs), a cell type commonly affected in CNS injury and disease, e.g. amyotrophic lateral sclerosis (ALS). MNs expressing eGFP under the Hb9-promoter were generated by directed differentiation of mouse embryonic stem cells and transplanted to the mouse hypoglossal nucleus by stereotaxic technique. Graft survival and integration was assessed at 1, 2 and 4 weeks post transplantation to uninjured animals, and at different time points after hypoglossal nerve injury. Graft survival was poor at early time points after hypoglossal nerve injury, correlating with high glial reactivity. However, we determined an optimal window for transplantation at a later time point. Local integration was assessed by analyzing synaptic inputs onto grafted cells. We measured synaptic covering using immunohistochemical markers for cholinergic (VAChT), glutamatergic (vGLUT2) and GABAergic/glycinergic (VIAAT) terminals. The composition of excitatory and inhibitory synapses was compared to that of resident MNs. We detected formation of glutamatergic and inhibitory synaptic terminals on grafted MNs as early as 1 week post transplantation, and increased at later time points. However, cholinergic terminals were not observed throughout the study. In terms of long range integration, we detected GFP-positive axons in the hypoglossal nerve and we are now

in the process of staining for neuromuscular synapses derived from the graft. Assessment of cortical input to the graft is ongoing. In summary, we present a new mouse model for studying engraftment of transplanted stem cell-derived MNs. By employing different methods for stimulating survival and integration, we hope to improve overall outcome for transplanted neurons, as a first step towards cellular therapy in a clinical setting.

Funding Source: Swedish Wenner-Gren Foundations. Vetenskapsrådet. Karolinska Institutet. Stiftelsen för strategisk forskning (SSF). Torsten och Ragnar Söderbergs stiftelser

NEURAL DISEASE AND DEGENERATION

W-1132

PHOSPHOLIPASE D3 CONTRIBUTES TO ALZHEIMER'S DISEASE RISK VIA DISRUPTION OF AMYLOID-BETA CLEARANCE THROUGH THE LYSOSOME

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Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β (Aβ) in the brain. We recently identified several coding variants in the phospholipase D3 (PLD3) gene that double the risk for late onset AD. PLD3 A442A was predicted to disrupt a splicing enhancer binding site such that splicing is reduced. We confirmed this splicing defect in human brain tissue from PLD3 A442A carriers. Human fibroblasts from a PLD3 A442A carrier were reprogrammed into induced pluripotent stem cells (iPSC). We then used CRISPR/Cas9 to correct PLD3 A442A using a seamless design. Differentiation of the isogenic pairs into cortical neurons produced cells that were morphologically similar. At the molecular level, the PLD3 A442A neurons displayed a similar defect in PLD3 splicing as was observed in human brains and a significant increase in Aβ levels compared with isogenic control lines. Thus, these findings illustrate that the PLD3 risk variant is sufficient to alter PLD3 splicing and Aβ metabolism. While the normal function of PLD3 is poorly understood, PLD3 is highly expressed in neurons and in brain regions most susceptible to amyloid pathology. PLD3 expression is significantly lower in AD brains compared with controls, and PLD3 expression is highly correlated with expression of lysosomal genes. Thus, we sought to determine whether PLD3 contributes to Aβ accumulation in AD via disrupted Aβ metabolism. We found that overexpression of PLD3 in immortalized cells decreased Aβ levels while shRNA silencing of PLD3 increased Aβ levels. In an AD mouse model, overexpression of PLD3 in hippocampal neurons produced decreased interstitial fluid (ISF) Aβ levels and accelerated Aβ turnover. Conversely, silencing of endogenous PLD3 increased ISF Aβ and reduced Aβ turnover. We hypothesize that reduced turnover of ISF Aβ leads to Aβ accumulation. To begin to determine whether PLD3 influences Aβ turnover via lysosomal function, we isolated lysosomal fractions from human AD and control brains. We found that PLD3 was enriched in lysosomal subfractions and that PLD3 distribution in these subfractions was altered in AD. Furthermore, PLD3 stability in the lysosomal fractions was disrupted in AD brains. Together, these findings demonstrate that PLD3 may contribute to AD risk via altered Aβ clearance through the lysosome.

W-1134

AMYLOID-B DEGRADATION IN CORTICAL NEURONS DERIVED FROM ALZHEIMER'S DISEASE IPSCS

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In familial forms of Alzheimer's disease (AD), increased production of amyloid-β (Aβ) is linked with Aβ deposition. However, the majority of AD patients are sporadic cases, where it is hypothesised that impaired clearance of Aβ may be the more important cause. These cases are typically late-onset, and aging remains the largest risk factor. This study aimed to assess Aβ degradation in different iPSC-derived neuron lines, where treatment with cell stressors may better model aging and sporadic AD. A method of assessing Aβ degradation has been adapted for use with cell lysates and intact cells in vitro. Fluorescently tagged Aβ (FAM-Aβ-Biotin) was incubated with cortical neurons (>60 days after neural induction) from healthy control and AD patient iPSC lines with and without hypoxic conditions to induce cell stress. At the end of the incubation with FAM-Aβ-Biotin, streptavidin coated dynabeads were added and uncleaved FAM-Aβ-Biotin separated by magnetic force. The supernatant containing the cleaved FAM-Aβ was measured. The assay established demonstrated that the Aβ-degrading metalloendopeptidases neprilysin (NEP) and insulin-degrading enzyme (IDE) can degrade the FAM-Aβ-Biotin substrate. Aβ degradation was then assessed in the iPSC-derived cortical neuron lines with and without hypoxic conditions and have indicated differences in their capacity to degrade Aβ. The addition of the protease inhibitor phosphoramidon, which is specific for NEP, indicates that this enzyme does not contribute significantly to Aβ degradation in

vitro. These data suggest that the various AD related iPSC-derived cortical neurons have different capacity to degrade Aβ that can also be altered by cell stress. Further investigation into how Aβ degradation in iPSC-derived neurons can be affected by the addition of external factors such as other cell stressors may better model ageing and sporadic AD.

W-1136

HUMAN GMP GRADE NEURAL STEM CELL TRANSPLANTATION RESCUES DEFICITS IN HUNTINGTON'S DISEASE MICE

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Huntington's disease (HD) is an inherited neurodegenerative disorder with no disease modifying treatment. Expansion of the glutamine-encoding repeat in the Huntingtin (HTT) gene causes broad deleterious effects challenging single treatment strategies. Effective strategies based on human stem cells offer promising long-term options. We transplanted a Good Manufacturing Practice (GMP) grade, human embryonic stem cell derived, human neural stem cell (hNSC) line into striatum of R6/2 HD model mice and found improvements in motor deficits. Extended studies demonstrated that implanted hNSCs are electrophysiologically active and rescue synaptic alterations. hNSCs also improved motor and late stage cognitive impairment in another HD model, Q140 knock-in mice. Disease modifying activity is suggested by reduction of the aberrant accumulation of mutant HTT protein and production of BDNF in both models, as well as increased synaptophysin and reduced neuroinflammation in Q140 mice. These findings hold promise for future pre-clinical and clinical development.

Funding Source: CIRM

W-1138

DEVELOPING AN IN VITRO MODEL OF SENSORY HAIR CELLS USING DIRECT CELLULAR REPROGRAMMING

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Hearing loss affects 360 million people worldwide and the primary cause is the loss of sensory hair cells in the cochlea. Hair cells are few in number and extremely fragile, making studies difficult. Here we have used direct lineage conversion to generate hair cell like cells in vitro. Our results demonstrate that a specific set of hair cell transcription factors is sufficient for reprogramming mouse and human somatic cells towards a hair cell fate. The induced hair cells (iHCs) express the respective fluorescent reporters, mouse Atoh1::nGFP or human Brn3c-TdTomato, as well as markers of primary hair cells, Myosin VI, Myosin VIIa, Phalloidin, Otoferlin and KCNQ4. The functionality of the iHCs has been assayed by their ability to take up Styryl dyes in a similar manner as primary hair cells. These results imply the proper expression and function of the ion channels believed to be responsible for the rapid entry of these compounds into primary hair cells. Whole cell patch clamping was also used to assay functionality and the iHCs repeatedly demonstrate positive outward currents that have activation kinetics characteristic of primary hair cell currents. We have conducted experiments to test the ototoxic vulnerability of iHCs. Ototoxins are compounds that have been implicated in hair cell loss. One known ototoxin is gentamicin, an aminoglycoside antibiotic, which is used in the clinic to treat severe infections. We performed a longitudinal survival tracking experiment with iHCs to assay for gentamicin-induced cell death. Time-lapse data was used to quantify the survival of iHCs following treatment. The results demonstrated a clear dose-dependent loss of iHCs in response to gentamicin. A preliminary otoprotectant screen was performed on iHCs treated with gentamicin. The screen examined 640 compounds for their ability to rescue the iHC survival deficit previously identified. The screen gave rise to 5 potentially protective compound that rescued the survival of iHCs by greater than 3 standard deviations from the mean survival of gentamicin treated controls. These compound hits are involved in known pathways of hair cell degeneration, namely the JNK, NF-kB, and DNA damage repair pathways. In Vitro mammalian iHCs will help us pursue protective and regenerative initiatives for the vulnerable sensory hair cells of the cochlea.

W-1140

CHARACTERIZATION OF TSC2 DEFICIENT HUMAN IPSC DERIVED PURKINJE CELLS IN AN IN VITRO MODEL OF AUTISM

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Tuberous sclerosis complex (TSC) is a neurodevelopmental disorder causing cortical tuber

formation, epilepsy and autism. TSC1/2 mutations dysregulate the mechanistic target of rapamycin (mTOR) pathway and disturb neuronal protein synthesis, which leads to the development of ASD approximately in 50 % of all TSC-patients. Previous PET imaging studies have indicated that cerebellar deficits, such as cerebellar lesions and glucose hypermetabolism in deep cerebellar nuclei, are associated with a higher prevalence of autism in TSC-patients. However, the exact cellular deficits that occur during development of autism hasn't been investigated in detail with patient derived neural cells. We have previously created a mouse model lacking the Tsc1 gene specifically in cerebellar Purkinje cells (L7-Cre+;Tsc1f/f). This mouse model displayed autistic-like behavioral deficits including increased repetitive behaviors, decreased social interaction and cellular abnormalities (Tsai et al, 2012, Nature). Together, the clinical findings from TSC-patients and the phenotype of the Tsc1 conditional knock-out mice indicate that cerebellar Purkinje cells have a crucial role in the development of ASD. To characterize the disease phenotypes of TSC at the cellular and molecular level we have developed a novel differentiation protocol for generation of Purkinje cells from human iPSCs with patient specific TSC2-mutations. In this study, we derived pluripotent stem cell lines from three TSC-patients and four unaffected familial controls or age-matched controls for neuronal differentiation in vitro. We find that TSC2-deficient patient iPSC-derived PCs have mTOR-pathway hyperactivation that was detected with increased levels of phospho-S6 and phospho-S6 kinase. Our comprehensive RNA sequencing analyses revealed several interesting genes related to neuronal differentiation deficits of TSC2-mutant iPSC-derived Purkinje cells. We also detected deficits in the electrophysiological properties of hiPSC-derived PCs. According to our data, inhibition of mTOR-pathway may improve the electrophysiological properties of TSC2-deficient human Purkinje cells, and could be a target for pharmacotherapy for treatment of ASD in TSC.

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W-1142

UTILIZING AN INDUCIBLE CRISPR/CAS9 PLURIPOTENT STEM CELL SYSTEM TO IDENTIFY CRITICAL RECEPTORS FOR ZIKA VIRUS INFECTION OF THE DEVELOPING HUMAN BRAIN

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The rapid spread of the Zika virus (ZIKV) in South and Central America has led to a resulting surge in microcephaly cases in newborn infants. Due to the severity and long-term impact of this disease, it is imperative that researchers develop treatments and vaccines as quickly as possible, and a better understanding of ZIKV tropism is a key step in this process. ZIKV infection, like other viruses in the Flaviviridae family, has been strongly linked to the presence of the TAM receptors: TYRO3, AXL, and Mer. Additionally, it has been shown that neural progenitor cells (NPCs) and astrocytes are particularly vulnerable to infection both in vivo and in vitro. Single-cell and population sequencing of neuronal differentiation models have indicated that AXL expression peaks substantially during the early NPC stage, which caused many to hypothesize that this is an essential route of entry for the virus into the developing brain. Through a dox-inducible CRISPR/Cas9 induced pluripotent stem cell (iPSC) system, we were able to quickly produce AXL-/- NPCs in 2D and 3D, and showed that these cells are equally vulnerable to ZIKV as isogenic wildtype controls. In 2D NPCs, both lines showed similar infectivity, cell death, and active viral RNA replication. Infected cerebral organoids from both lines showed similar rates of size reduction, cell death, and cleaved caspase-3 upregulation. These results are of clinical importance, indicating that AXL inhibitors may not be sufficient to prevent ZIKV infection of NPCs, as well as other cell types with a likely expression of alternative anchoring receptors. Further investigation into early expression of these TAM receptors has indicated that TYRO3 expression peaks during NPC differentiation much like AXL, though with a higher baseline expression. Additionally, recent studies have shown that astrocytes, which lack TYRO3 expression, appear to respond to antibody blocking of AXL. These combined observations indicate that ZIKV may be capable of utilizing either AXL or TYRO3 to anchor to vulnerable cell types. Utilizing the accelerated rate at which the dox-Cas9 iPSC system can produce knockouts, as well as a new method for rapidly producing screenable NPCs, we have developed AXL-/- TYRO3-/- double knockouts to discover if these

receptors are truly critical for ZIKV infection of the developing brain.

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W-1144

BONE MARROW MESENCHYMAL STROMAL CELLS FROM SPORADIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS EXHIBIT DECREASED IMMUNOMODULATORY AND TROPHIC FUNCTIONS AND ALTERED NON-CODING RNA EXPRESSION

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of cortical and spinal motor neurons. Cell therapy using mesenchymal stromal cells (MSCs) has been proposed as a promising therapeutic approach for the treatment of various neurological diseases including ALS. MSCs can be obtained from autologous (bone marrow (BM) and adipose tissues) or from allogeneic sources (umbilical cord and placenta) and these cells exhibit immunomodulatory potential, migratory capacity to injured areas and paracrine regenerative effects. Although phenotypic and functional changes have been reported in MSCs derived from patients with different diseases, the characteristics of MSCs from ALS patients are not fully understood. Here we compared the secretome, gene, miRNA expression and exosome secretion of MSCs derived from sporadic ALS (sALS) and healthy controls. We found that MSCs from ALS patients exhibited similar mesenchymal differentiation abilities and a transient higher growth rate in culture. Using gene array analysis we found that MSCs obtained from sALS patients demonstrated a decrease in the expression of genes associated with angiogenesis, cardiovascular system development, cell migration, adhesion, morphogenesis, chemotaxis, wound healing and muscle system processes. In contrast, pathways associated with mitotic cell cycle, DNA metabolic processes, protein kinase A signaling and E2F4 targets were upregulated in these cells. We also found that MSCs obtained from sALS patients expressed lower levels of SOCS1 and IL-10 that are associated with the anti-inflammatory effects and lower levels of TGF-beta2, IGF-1 and VEGF that are associated with trophic and regenerative capabilities of these cells. MSCs obtained from sALS patients also varied in the levels and miRNA content of secreted exosomes compared with cells secreted from healthy donors. In summary, the major phenotypic and functional changes observed in this study may provide an important information regarding the role of endogenous BM-MSCs in various stages of ALS initiation and progression, contributes to the identification of novel disease markers and have important implications for the use of autologous cells for cell therapy in ALS and other neurodegenerative diseases.

W-1146

HUMAN ESC-DERIVED MSC PROMOTE RECOVERY OF MONKEYS FROM EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Non-human primate experimental autoimmune encephalomyelitis (EAE) is a valuable model for evaluation and translation of new therapies for inflammatory demyelinating diseases. We have previously demonstrated that human embryonic stem cell (hESC) derived mesenchymal stem cells (hES-MSC) are efficacious in treatment of a murine EAE model. However, it remains unknown whether the efficacy of hES-MSC is translatable to humans. In this study, we induced EAE in a non-human primate species cynomolgus monkeys (Macaca fascicularis) via injection of a peptide from human myelin/oligodendrocyte glycoprotein (MOG35-55) and complete Freund's adjuvant. Magnetic resonance imaging and clinical evidence show that EAE was successfully induced in all seven injected monkeys. We administrated some of the animals through intrathecal infusion of hES-MSC at 10 million cells/time for three times post-onset and upon relapses. Clinical symptoms relieved rapidly within 3 days post the cell injection. During follow-up for three months, disability score in the treated monkeys declined gradually although some low-degree relapses occurred. No tumor was found in isolated monkey organs and tissues. Thus, our results suggest that hES-MSC administered intrathecally are effective and safe in

the treatment of EAE in monkeys, a promising evidence to advance the therapy to humans.

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W-1148

INTERROGATING INTRINSIC MICROGLIAL ABNORMALITIES AND THEIR FUNCTIONAL IMPACT ON NEURONS USING ISOGENIC HUNTINGTON DISEASE HUMAN PLURIPOTENT STEM CELLS

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Microglia are resident macrophages in the central nervous system and constitute about 10% of the total cells in the adult brain. Under normal conditions, microglia contribute to the maintenance of tissue homeostasis and are also involved in synaptic plasticity and learning. Conversely, microglia have been implicated in a number of brain conditions, including Huntington disease (HD), a progressive neurodegenerative disorder and the most common genetic cause of dementia. HD is caused by a polymorphic mutation in exon 1 of the huntingtin (HTT) gene, namely a CAG repeat expansion whose length correlates inversely with the rate of clinical onset. While the evidence points to HD microglia being hyperactive, the impact of this abnormal activation on neurons and its relationship to disease onset remains poorly defined. Here we sought to develop a human pluripotent stem cell (hPSC)-based model system to address this issue. First, I established and refined methods to differentiate hPSCs into macrophages. The hPSC-derived cells expressed canonical macrophage markers including CD11b, CD14, and CD163 and exhibited bona fide macrophage functions such as efficient phagocytosis and robust secretion of inflammatory mediators upon activation. Second, I established long-term co-cultures of macrophages with neurons derived from hPSCs. Longitudinal imaging analysis revealed the macrophages to behave in microglia-like manner, interacting with the neurons and in particular the axonal extensions. Using this system and a novel panel of isogenic HD (IsoHD) hPSCs harbouring HTT alleles with different CAG repeat expansions, I am examining HD hPSC-derived microglia and their impact on neuronal health and function. Specifically, I am examining whether HD hPSC-derived microglia (a) exhibit intrinsic functional abnormalities and (b) have adverse effects on neuronal health and function. Finally, I will examine the extent to which any of the microglial abnormalities correlate with CAG repeat length and, by extension, possibly contributor to the rate of disease progression. We anticipate that our study using IsoHD hPSC-based approach will provide new insights into the role of microglia in HD and will shed light on neuroinflammation as a pathogenic factor for neurodegenerative disorders in general.

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W-1150

FROM YEAST TO NEURONS TO PATIENTS: PROTEOME-SCALE DISSECTION OF PROTEINOPATHY FOR TARGETED TREATMENT OF NEURODEGENERATION

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It is unclear how diverse genetic risk factors for neurodegenerative diseases relate to the misfolding of specific proteins that characterize their neuropathology. Alpha-synuclein is a small lipid-binding protein that misfolds in diverse neurodegenerative diseases known as synucleinopathies. These include Parkinson's disease, multiple system atrophy and dementia with Lewy bodies. Currently, there are no therapies targeting alpha-synuclein-induced cellular pathologies. We have recently developed a suite of experimental and computational approaches to model and target alpha-synuclein toxicity in cellular systems, ranging from simple yeast cells to complex patient-derived stem-cell models. We have now employed unbiased proteome-scale screens to assemble molecular networks comprised of genetic and physical interactors of alpha-synuclein. These approaches have linked alpha-synuclein proteotoxicity to diverse genetic risk factors for parkinsonism through specific molecular pathways. We have been able to predict convergent pathologies in pluripotent stem cell-derived neurons from patients with diverse forms of parkinsonism, and identified small-molecules capable of reversing the toxicity in these models. Targeted exome sequencing of genes in our molecular alpha-synuclein network has revealed putative novel genetic risk factors for Parkinson's disease, and we present approaches being pursued for functional validation in our cellular models. Thus, proteome-scale cellular screens combined with computational network approaches, stem-cell models and human genetic analysis offer promising

approaches to stratify patients and target treatments according to molecular mechanisms.

Funding Source: JPB Foundation, Howard Hughes Medical Institute, NIH, Multiple System Atrophy Coalition, American Brain Foundation, Parkinson's Disease Foundation

W-1152

GENERATION AND PHENOTYPE CHARACTERIZATION OF AD PATIENT-SPECIFIC IPSC LINES

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Alzheimer's disease (AD) is the most common neurodegenerative disease which is pathologically characterized by the formation of amyloid-beta (Aβ) plaques and neurofibrillary tangles. Recent studies have demonstrated that excessive accumulation of Aβ peptides can increase hyperphosphorylation of tau, leading to the formation of neurofibrillary tangles. In this study, we have generated several induced pluripotent stem cell (iPSC) lines from familial and sporadic AD patients, and then differentiated them into cortical neurons. Extracellular deposition of Aβ levels were dramatically increased in the neurons differentiated from iPSC lines generated from the patients carrying presenilin-1 (PS1)-S170F mutation, amyloid precursor protein (APP)-V715M mutation or sporadic AD. Furthermore, some of these AD iPSC-derived neurons exhibited high expression levels of phosphorylated tau, especially in AT8 (Ser202/Thr205), which are also detected in the soma and neurites by immunocytochemistry. We next investigated the mitochondrial dynamics in AD iPSC-derived neurons, which exhibited abnormal patterns of mitochondria velocity, and fission and fusion process using Mito-tracker. We also found that the levels of Mfn1 (membrane proteins mitofusin 1) and Mfn2 (membrane proteins mitofusin 2) were significantly reduced in AD iPSC-derived neurons. We also observed that LC3b and ubiquitin is highly increased in AD iPSC-derived neurons, indicating that the autophagy system is also defective. Taken together, we have characterized the pathological features of AD patients carrying mutations for PS1-S170F or APP-V715M using iPSC technology for the first time, which will serve as useful resources for studying AD pathogenesis and drug screening in the future.

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W-1154

A HIGH-THROUGHPUT DISEASE-SPECIFIC PHENOTYPE DETECTION SYSTEM OF PARKINSON'S DISEASE FOR DRUG SCREENING

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Disease-specific induced pluripotent stem cells (iPSCs) established from one of the familial Parkinson's diseases (PDs), PARK2, showed impaired mitochondria clearance (Mol. Brain 2012) due to the impaired function of the parkin gene. To perform efficient drug screening using dopaminergic neurons differentiated from PARK2-iPSCs, we sought to establish a 96-well based high-throughput neural differentiation and phenotype detection system. PARK2-iPSCs were differentiated into dopaminergic neurons on the 96-well plates by neurosphere based differentiation protocol (Stem Cell Reports 2016). Then, several PD-related phenotypes, including impaired mitophagy, accumulation of ROS, and increased apoptosis were quantified automatically by using In Cell Analyzer 2200 and In Cell Developer Toolbox (GE healthcare). By using this high-throughput assay system, we performed library screening (320 compounds) evaluating phenotypic recovery. We identified several compounds that improve multiple phenotypes observed in PARK2 dopaminergic neurons. This high-throughput neural differentiation of patient specific iPSCs and phenotype detection system is an effective tool for drug screening to explore disease-modifying drugs in PD.

W-1156

AUTOPHAGIC AND LYSOSOMAL ACTIVITIES IN LRRK2 OR GBA1 MUTANT IPSC-DERIVED MIDBRAIN DOPAMINERGIC NEURONS

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In this study we aim to better understand the dysfunctional changes in autophagic pathways and α-Synuclein accumulation caused by known genetic mutations related to Parkinson's disease (PD). The subjects included 3 healthy donors, 4 PD patients carrying heterozygous LRRK2 (leucine-rich repeat kinase 2) G2019S mutation, and 2 individuals with heterozygous GBA1 (glucocerebrosidase) N370S mutation. We successfully differentiated and isolated iPSC-derived mid-brain dopaminergic (mDA) neurons and systematically characterized the cellular changes of these cells. We showed a significant increase of monomeric α-Synuclein in genetically affected neurons. This result suggested that GBA1 and LRRK2 mutant neurons failed to degrade α-Synuclein in a timely manner. To tease out the probable cause to this defect, we investigated the function of chaperone-mediated autophagy (CMA) and macroautophagy. Photo-convertible CMA reporter was employed to assess the general CMA activity. LRRK2 mutant mDA neurons had impaired CMA activity. Consistently, the numbers of intracellular foci of CMA receptor Lamp2A were decreased in LRRK2 mutant neurons. Interestingly, the Lamp2A expression levels were increased in GBA1 mutant mDA neurons while that of LRRK2 mutant was decreased. The defects in CMA were accompanied by an increased LC3 flux in both LRRK2 and GBA1 mutant mDA neurons. Our results suggested different regulation of the CMA pathway by LRRK2 and GBA1. Dysregulated CMA might result in a compensatory mechanism of elevated macroautophgy.

CANCERS

W-1160

CANCER STEM CELL PROLIFERATION IN HUMAN PROSTATE AND BREAST CANCER CELL LINES UTILIZING A NEW DEFINED SERUM-FREE 3D CANCER STEM CELL MEDIA

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Solid tumors grow in a three-dimensional (3D) spatial conformation, resulting in a heterogeneous exposure to oxygen and nutrients as well as to other physical and chemical stresses. To mimic the 3D spatial conformation, 3D in vitro culture models have been used in cancer research since the diffusion-limited distribution of oxygen (hypoxia), nutrients, metabolites, and signaling molecules is not mimicked in conventional two-dimensional (2D) monolayer cultures. Cancer stem cell (CSCs) are defined as a small subset of cells within a tumor with the ability to self-renew and often drive tumor progression and recurrence after chemotherapy treatment. Traditionally, cancer stem cells have been isolated from cancer cell lines and tumor biopsies and grown in undefined serum containing media using 3D tumorsphere cultures. Here we report a new defined serum-free cancer stem cell media that supports the expansion of human prostate (E006AA) and breast (MCF-7) cancer cell lines using a 3D tumorsphere culture method. Both cells showed continuous proliferation supported during serial passage of 3D tumorsphere cultures. In addition to the stable proliferation, increased Aldehyde Dehydrogenase (ALDH) expressing cancer stem cell populations was observed after multiple passages in this new 3D cancer stem cell media.

W-1162

USING PLURIPOTENT STEM CELLS TO IDENTIFY EMBRYONIC-MECHANISMS MEDIATING AGGRESSIVE BREAST CANCER

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Reliable approaches to identify and target stem-cell mechanisms that mediate aggressive cancer could have great therapeutic value, based on the growing evidence of stem-like signatures in metastatic cancers. However,

how to best identify and target stem-like mechanisms aberrantly acquired by cancer cells has been challenging. We harnessed the power of iPSCs to identify embryonic mechanisms exploited by cancer. A screen comparing the cell surface proteome of iPSCs and breast cancer cells identified GRP78, a protein which is normally ER-restricted, but which has been shown to be aberrantly expressed on the cell surface of several cancers, where it can act as a signaling molecule by poorly understood mechanisms. Although cell surface GRP78 (sGRP78) has emerged as an attractive chemotherapeutic target, understanding how sGRP78 is functioning in cancer has been complicated by the fact that GRP78 can function to regulate a variety of cellular responses, using a diverse array of reported binding partners, which can vary by cell type. Therefore, without insight into the specific GRP78-dependent mechanisms that are responsible for specifically mediating aggressive cancer, it will be difficult to determine how to best target GRP78. We have discovered that (1) sGRP78 is expressed on iPSCs (but not their somatic parental populations) and plays an important role in reprogramming, (2) sGRP78 promotes cellular functions such as proliferation/survival and migration in both stem cells and breast cancer cells (3) overexpression of GRP78 in breast cancer cells leads to an induction of a previously established CD24-/CD44+ 'cancer stem cell' (CSC) population (4) sGRP78+ breast cancer cell populations are enriched for stem-like genes and appear to be a subset of previously established CSCs (5) sGRP78+ breast cancer cell populations show a significantly enhanced ability to seed metastatic organ sites in vivo. These collective findings suggest that sGRP78 marks a stem-like population in breast cancer cells that has increased metastatic potential in vivo. We are currently identifying the sGRP78-mediated mechanisms utilized by cancer that are common to iPSCs, to reveal the specific sGRP78 stem-like mechanisms that lead to the most aggressive cancer outcomes, critical for potential future therapeutic targeting of sGRP78.

W-1164

RECRUITING THE TIP60-P400 COMPLEX TO MAX/DNA IS REQUIRED FOR IPSC GENERATION AND INVOLVED IN TUMORIGENESIS

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The Tip60-p400 histone acetyltransferase (HAT) complex, also called human NuA4 complex, is involved in multiple biological events including DNA damage response, stem cell maintenance, interaction with factors relevant to tumorigenesis. Several Tip60-p400 complex subunits, including the common HAT complex subunit TRRAP, have been reported to bind c-Myc. However, the scaffold protein EP400 is the only subunit not also found in distinct complexes. Therefore, it is still inconclusive if c-Myc recruits the intact Tip60-p400 complex to c-Myc target genes, if this transcription activator and HAT complex interaction is the causal factor of any human cancer, and if it contributes to the reprogramming activity of MYC in induced pluripotent stem cell (iPSC) generation. Merkel cell carcinoma (MCC) is an aggressive neuroendocrine skin cancer frequently containing integrated Merkel cell polyomavirus expressing small T antigen (ST), which has been implied as the causal factor of MCC tumorigenesis. From multi-dimensional protein identification technology (MudPIT) analysis, we found that ST antibody enriched a Tip60-p400 complex containing L-Myc and MAX as stable subunits instead of weakly associated transcription coactivators. We further demonstrate that ST bridges the stabilized interaction between L-Myc/Max and TRRAP. Disruption of the L-Myc-ST-p400 complex led to decreased MCC cell proliferation and increased levels of differentiation associated genes. Wild type ST, but not a Tip60-p400 binding mutant, could coordinate with L-Myc in transforming primary human cells. MYC interaction with the Tip60-p400 complex has been implicated in maintenance of ESC and iPSC. However, previous study using c-Myc mutants deficient in TRRAP binding suggested that TRRAP might not be involved in c-Myc induced iPSC generation. Our data argue that Myc family proteins and their mutants, unlike ST, are weak interacting coactivators not sensitive for analyzing biological functions of TRRAP binding. In fact, we demonstrate that wild type ST, but not a Tip60-p400 binding mutant, can replace L-Myc and cooperate with OCT4/SOX2/KLF4 to generate iPSC. Therefore, ST provides insight into the biological functions of otherwise elusive MAX/DNA-p400 HAT complex interaction mediated by MYC family members in tumorigenesis and reprogramming.

W-1166

THE ENHANCED SELF-RENEWAL CONFERRED ON HSPCS BY COHESIN HAPLOINSUFFICIENCY CAN BE REVERSED BY DIMINISHING HOXA7 AND HOXA9 GENE EXPRESSION USING DOT1L INHIBITORS

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Acute myelogenous leukemia (AML) is a high-risk hematopoietic malignancy with a poor prognosis. Developing novel treatments for AML has been difficult due to the heterogeneity of genetic mutations observed across patients. Recent studies have demonstrated that haploinsufficiency of the cohesin complex leads to enhanced self-renewal in Hematopoeitic Stem and Progenitor Cells (HSPCs). We sought to delineate the molecular mechanisms by which cohesin mutations promote enhanced HSPC self-renewal and to identify possible therapeutic interventions to reverse the phenotype conferred on HSPCs by cohesin haploinsufficiency. In the present study we observed that reduced cohesin levels elicit enhanced self-renewal of murine HPSCs in vitro in serial-replating assays. Gene arrays and RT-qPCR identified significant increases in the HSPC self-renewal factors Hoxa7 and Hoxa9. Rad21 depletion resulted in decreased levels of H3K27me3 at the Hoxa7 and Hoxa9 promoters, indicating that Rad21 plays a role in proper PRC2-mediated silencing of these genes. Using immunoprecipitation experiments we further demonstrate that cohesin and PRC2 directly interact and are bound in close proximity to Hoxa7 and Hoxa9. Consistent with this finding we observed reduced global levels of PRC2's repressive histone mark H3K27me3 arguing that PRC2 requires the cohesin complex for proper targeting. Importantly, knockdown of either Hoxa7 or Hoxa9 suppressed self-renewal, implying both are critical downstream effectors of reduced cohesin levels. Given the established role of Dot1l in regulating Hoxa9 gene expression by depositing the histone mark H3K79me2, we sought to evaluate whether decreasing Dot1l function reverses the phenotype caused by cohesin haploinsufficiency. Interestingly, inhibition of Dot1l function reverses the enhanced self-renewal capacity of cohesin haploinsufficient HSPCs. Consistent with our proposed mechanism, this reduced self-renewal occurred concomitant with a reduction in Hoxa7 and Hoxa9 gene expression. Our data demonstrate that the cohesin complex regulates PRC2 targeting to silence Hoxa7 and Hoxa9 and negatively regulate self-renewal and establishes feasibility for the use of Dot1l inhibition as a targeted therapeutic treatment for AML patients harboring cohesin mutations.

W-1168

EARLY EXPOSURE TO ELEVATED IGF-1 LEVELS EXPANDS MAMMARY STEM CELL COMPARTMENT AND INCREASES MAMMARY TUMOR SUSCEPTIBILITY

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Breast cancer (BrCa) is the most common cancer in women. Despite substantial research, BrCa incidence continues to rise, and outcome disparities persist. African American (AA) women suffer higher mortality than White Caucasians. Assessment of this disparity reveals that AA women are more likely to develop the early onset, treatment refractory, triple negative BrCa subtype associated with worse prognosis. The mechanistic basis for the difference in development of BrCa subtypes remains unresolved, but it has been shown that young AA girls have significantly higher circulating levels of Insulin-like growth factor 1 (IGF-1) than their age-matched White counterparts, implicating early exposures to this mitogenic and pro-tumorigenic growth factor in mediating BrCa subtype. To investigate the role of IGF-1 in mammary tumorigenesis, we use the transgenic (Tg) BK5.IGF-1 model, which recapitulates the paracrine effects of IGF-1 exposure on the mammary epithelium. We previously showed that exposure to elevated levels of IGF-1 are strongly pro-tumorigenic in the mammary gland, and pre-pubertal Tg mice have an increased number of terminal end buds, which are known to be important stem cell niches. In this study, we found that the mammary stem cell (MaSC) pool was expanded in both pre- and post-pubertal Tg mice compared to age-matched WT animals. Flow cytometry and immunolocalization identified the expression of IGF-1R on both WT and Tg MaSCs. Single-cell transcriptomic analysis of MaSC compartment revealed that IGF-1 stimulated Cyclin D1 and Cyclin G2 gene expression and increased the proliferation of "activated" transient stem cells (T-MaSCs). Moreover, Gene Set Enrichment Analysis demonstrated that genes involved in stemness, proliferation, EMT, invasion and metastasis were highly upregulated in T-MaSCs from Tg mice compared to age-matched WT animals. Interestingly, GO enrichment analysis also showed downregulation of genes associated with cell polarity in T-MaSCs from Tg animals, suggesting an increased number of stem cells undergoing symmetric cell divisions. Overall, our results identify a novel tumorigenic mechanism, by which early exposure to IGF-1 expands the MaSC compartment and "primes" these cells for transformation, thereby

increasing mammary tumor incidence and reducing latency.

W-1170

THE NOVEL ROLE OF THE DEVELOPMENTAL REGULATOR SALL4 IN MELANOMA TUMORIGENESIS

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During embryonic development, Neural Crest Stem Cells (NCSCs) gives rise to many neuronal cell types of the peripheral nervous system, as well as to non-neuronal cell types, amongst others the melanocytes. Cutaneous melanoma, which originates from the melanocytes of the skin, is one of the most aggressive cancers. Melanoma cells can in fact often metastasize to proximal and distant organs and their invasive capacity is to some extents reminiscent to the great migratory and invasive properties of the neural crest (NC) cells. This has contributed to the hypothesis that NC-derived malignant cells may exploit developmental regulatory programs to gain an advantage over normal non- malignant cells and to progress to more advanced stages of the disease. The transcription factor Sall4 is a well-known developmental regulator involved in embryonic patterning, limb formation and embryonic stem cell maintenance but only little is known about its role in NC biology. An ongoing study in our lab had identified Sall4 to be downregulated in NCSCs which were isolated from mouse embryos, primed towards differentiation by exposing them to instructive growth factors, and compared to multipotent (non-primed) NCSCs. While the developmental role of Sall4 in the NC is still under investigation, we started to address its putative role in melanoma. To do that, we used the Tyr::NrasQ61KINK4a−/−Tyr::CreERT2Sall4lox/loxR26R::GFP murine model, which allows us to conditionally ablate Sall4 in the melanocytes of mice spontaneously developing melanoma. Interestingly, those mice having Sall4 ablation did not form primary tumors compared to the control animals but strikingly had more melanoma metastasis (GFP+ cells) in the lungs. With siRNA-mediated SALL4 knock down experiments in human cell lines in vitro, we could additionally verify that SALL4 decrease correlates with upregulation of melanoma invasiveness genes. Since SALL4 is known to act as an epigenetic regulator in development, we carried out Co-Immunoprecipitation experiments on human melanoma cell lines and could show that SALL4 binds to histone deacetylase 2 (HDAC2). We therefore hypothesize that SALL4 functions as epigenetic regulator to modify transcription of its target genes.

W-1172

MICROVESICLES DERIVED FROM NORMAL AND MULTIPLE MYELOMA BONE MARROW MESENCHYMAL STEM CELLS DIFFERENTIALLY MODULATE MYELOMA CELLS' PHENOTYPE AND TRANSLATION INITIATION

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Multiple myeloma cells' (MM) interaction with the bone marrow (BM) microenvironment critically hinders disease therapy. We have shown that co-culture of MM and BM-mesenchymal stem cells (MSCs) co-modulated translation initiation (TI) and cell phenotype with involvement of microvesicles (MVs). Here, we studied the effects of MVs derived from normal donors (ND) MSCs and MM-MSCs on MM cell lines' phenotype and TI. Particularly, we studied the MVs initial influence upon contact and later effect after uptake into MM cells. BM-MSCs were extracted, propagated and validated as previously (ND- femur heads and MM patients' aspirates). MVs were isolated from BM-MSCs secretomes. Bona-fide BM-MSCs' MVs were applied to MM cell lines, their uptake outlined, and recipient MM cells were assayed for viability; proliferation; death; migration; invasion; autophagy; TI status; MAPKs activation. The interdependence of MAPKs, TI, and autophagy was determined using inhibitors. The significance of MVs' cargo components (RNA, proteins) to MM cells' response was tested (RNase A, 65ºc). The role of integrins in initial contact induced MM cells response was tested (RGD). MVs protein cargo was assayed by mass spectrometry. ND-MSCs MVs' treated MM cells demonstrated a rapid (5min) activation of MAPKs followed by a persistent decrease (1-24h), while MM-MSCs MVs' treated cells demonstrated a continued activation of MAPKs and TI (5min-24h; ↑25-200%). Upon uptake (24h, 95%) ND-MSCs' MVs decreased viability, proliferation, migration, invasion and TI (↓15-80%), whereas MM-MSCs' MVs increased them (↑10-250%). Inhibition of MAPKs in MM-MSCs MVs treated MM cells decreased TI and inhibition of autophagy elevated cell death. Inhibition of the MM-MSCs MVs' uptake into MM cells diminished their effects. Denaturation/degradation of protein/RNA contents in MM-MSCs' MVs decreased their stimulation of proliferation and TI in recipient MM cell lines'. Higher integrin α4 in MM versus ND-MSCs MVs highlighted its possible role in MVs–MM contact and initial signaling. Indeed, RGD reduced the rapid MAPKs and TI activation in MM cells treated with MM-MSCs MVs. These results show that BM-MSCs MVs have

a two phase effect on MM cells' phenotype contingent on source and implemented via TI modulation.

CHROMATIN AND EPIGENETICS

W-1174

TWO CONSERVED HISTONE H3 LYS9 DEMETHYLASES KDM3A AND KDM4C REGULATE MSCS SENESCENCE PROGRESS

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Cellular senescence is a process in which cells experience the gradual loss of proliferation and differentiation potential. Senescence at cellular level contributes to organism aging. Bone Marrow Stromal Cells (BMSCs) are extremely important adult stem cells for tissue homeostasis, regeneration and repair. The regenerative function of BMSCs declines in aged people. Reciprocally, BMSC senescence contributes to geriatric diseases, such as osteoporosis and osteoarthritis. Previous work has shown that cellular senescence is accompanied by extensive spatial rearrangement of heterochromatin. However, the question of whether heterochromatin reorganization underlies BMSC aging and the mechanisms involved in initiating and maintaining this unique epigenetic state remain unclear. Here, we show that BMSC senescence is accompanied by a dynamic heterochromatin reorganization process. Moreover, we have identified two H3K9 demethylases, KDM3A and KDM4C, which are highly expressed in senescent BMSCs. Knockdown of KDM3A or KDM4C induces DNA-damage-induced cellular senescence whereas overexpression of KDM3A or KDM4C results in heterochromatin collapse and subsequent cellular senescence. Mechanically, we have revealed that KDM3A and KDM4C play critical roles in the assembly and disassembly of heterochromatin structure. The regulatory effects of KDM3A and KDM4C on heterochromatin remodeling are not only executed by their demethylase activity, but also by their scaffold function which enables the interaction with chromatin condenser proteins. In addition, increased expression of KDM3A/KDM4C is detected in BMSCs derived from both osteoporotic rats and aging mice. More importantly, the association of KDM3A/KDM4C and dynamic change of heterochromatin has been recapitulated in the osteoporotic rats. In conclusion, our study we have revealed a previously undefined role of histone demethylase in controlling MSC senescence. Moreover, the findings of our study provide novel insights into the chromatin reorganization that governs stem cell senescence and aging process.

W-1178

REVEALING THE DEVELOPMENTAL IMPLICATIONS OF GENOMIC IMPRINTING USING ANDROGENETIC AND PARTHENOGENETIC HUMAN EMBRYONIC STEM CELLS

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Parental genomes in mammals are functionally non-equivalent due to genomic imprinting, an epigenetic process that confers the parent-specific differential regulation of particular genes. Imprinting renders the co-contribution of maternal and paternal genomes indispensable for development, and its dysregulation is associated with human disease. Whereas imprinting has been extensively studied in the mouse, mechanistic investigation into human-specific aspects of this unique phenomenon has been challenging. To explore imprinting in humans at the molecular and functional levels, we utilized human embryonic stem cells (ESCs) with distinct parental origins. We derived three completely-paternal human androgenetic ESC (aESC) lines using sperm injection into human oocytes with removal of the maternal genome. This procedure resulted in efficient development to the blastocyst stage and subsequent derivation of pluripotent aESCs capable of self-renewal and differentiation. In addition, we analyzed completely-maternal parthenogenetic ESCs (pESCs) derived by activation of unfertilized oocytes, and bi-parental ESCs derived by in vitro fertilization (IVF-ESCs). Comparing the transcriptomes and methylomes of aESCs, pESCs and IVF-ESCs enabled the identification of known imprinted loci, as well as several putative imprinted genes at novel regions. We next harnessed human aESCs and pESCs to study parent-specific developmental biases. It has long been appreciated that the maternal and paternal genomes are developmentally biased towards the embryonic and extraembryonic lineages, respectively, as reflected by mouse uniparental embryos and human uniparental tumors. Differentiation into embryoid bodies in vitro and teratomas in vivo both recapitulated the tendency of androgenetic cells to contribute to the placenta, and remarkably, revealed another significant paternal bias towards liver differentiation. We therefore differentiated aESCs and pESCs into trophoblast cells and hepatocytes by directed differentiation, aiming to study the mechanistic roles of specific imprinted genes that may underlie these phenotypes. Our results demonstrate the potential of ESCs from different

parental origins for studying the impact of imprinting on human development and disease.

W-1180

ENDOGENOUS RETROVIRUSES UNDERLIE A PRIMATE-SPECIFIC GENE REGULATORY NETWORK IN HUMAN NEURAL PROGENITOR CELLS

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The complexity of human brain development differs markedly from other mammals and is thought be important for the emergence of higher cognitive functions. However, the precise genetic changes, as well as the existence of human-specific gene regulatory networks underlying the evolution of the human brain remains poorly explored. Most of our knowledge about human brain development is restricted to evolutionary conserved developmental pathways, while much less is known about primate- and human-specific developmental mechanisms. Identification of novel mechanisms that regulate human brain development is important for our understanding of the human brain and may also provide new links to the biology of human brain disorders. About 8% of the human genome is composed of endogenous retroviruses (ERVs). These sequences are derived from retroviruses that have invaded vertebrate hosts for millions of years leaving traces as inherited ERVs through germ line infection and subsequent transposition. Several studies have found that ERV transcription is tightly controlled at multiple levels in early human development and they have been proposed to participate in the control of gene regulatory networks. In this study, we find a region- and developmental stage-specific expression pattern of ERVs in the developing human brain, which is linked to a transcriptional network based on ERVs. We demonstrate that several thousand primate-specific ERVs act as docking platforms for transcriptional repression, either by attracting the epigenetic co-repressor protein TRIM28 or by recruiting DNA methylation. This results in the establishment of local heterochromatin around these ERVs. This repressive transcriptional network modulates expression of protein-coding transcripts important for brain development, thereby providing an additional layer of transcriptional regulation. Our findings open up for several exciting future studies on the role of ERVs as potential drivers of human brain evolution, their contribution to individual variation and the implication in human brain disorders.

ORGANOIDS

W-1184

A XENOGENEIC-FREE SYSTEM GENERATING FUNCTIONAL MINI-GUTS FROM HUMAN EMBRYONIC STEM CELLS

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Functional intestines are generated through a highly orchestrated, serial developmental process and are composed of cell types from all three primary germ layers. Directed differentiation of human pluripotent stem cells (hPSCs) can yield gut-specific cell types; however, these structures do not replicate some of the critical functional interactions between cell types of different germ layers. Here, we developed a simple protocol based on a tissue self-organization concept for generation of mature functional intestinal organoids from human embryonic stem cells (hESCs) under xenogeneic-free (XF) conditions. Cells were placed in XF differentiation medium and seeded on a circle-patterned dish. This surface was coated with the polyethylene glycol micropatterning substrate, which stimulated cell assemblage. By day 30, self-organized cystic spheroids began to detach from the plate, and some exhibited contractile movements. In the early differentiation phase, growing patterned cells expressed markers of definitive endoderm, FOXA2, SOX17, and CXCR4, and of early endoderm, GATA4 and GATA6; their relative levels of expression increased in the hemispheric dome-like structures at day14. The level of the hindgut marker CDX2 increased during early growth and was maintained at a relatively high level as differentiation progressed. During subsequent differentiation, cell accumulations formed cavitated structures and detached from the substratum. The organoids showed the gut tube-like architecture consisted of mucosa and submucosa by histological, immunofluorescence and electron micrograph examinations. Stem cell-derived functional gut organoids, mini-guts were derived from all three germ layers and contained distinct types of intestinal cells, including enterocytes, goblet cells, Paneth cells, and enteroendocrine cells. They demonstrated intestinal functions including peptide absorption and innervated bowel movements in response to stimulation with histamine and anticholinergic drugs. Our xenogeneic-free approach for generating mini guts from hESCs

provides a novel platform for studying human intestinal diseases and for pharmacological testing.

W-1186

LONG-TERM EXPANSION OF MOUSE HEPATIC STEM CELLS IN 3-DIMENSIONAL CULTURE USING HEPATICULT™, A SERUM-FREE HEPATIC ORGANOID EXPANSION MEDIUM

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Growing hepatocytes as organoids in a 3-dimensional (D) cell culture environment represents a more physiological model system than conventional 2-D adherent cell culture for studying many different aspects of liver cell biology. These hepatic organoids are spherical structures composed of a polarised monolayer of epithelial cells that retain many key features of hepatocytes in vivo (Huch et al, Nature 2013). We are currently developing HepatiCult™, a serum-free medium that promotes the generation of hepatic organoids from mouse liver tissue. Mouse livers were enzymatically digested to remove mature hepatocytes, and the resulting hepatic ducts were embedded in Corning® Matrigel® and cultured in HepatiCult™. Liver organoids can be visualised budding from nearly 100% of plated ducts within 24 hours after plating, thereby indicating the presence of a putative population of liver stem and progenitor cells. These hepatic organoids were then serially passaged every 5-7 days at 1:4 to 1:10 split ratios and maintained long-term for > 1 year, thereby indicating the presence of self-renewal of hepatic stem cells. Cells within organoids expressed Lgr5, Sox9, Keratin (Krt) 7, Krt19, EpCAM and Hnf4α, but not markers of mature hepatocytes; and can be induced to differentiate into mature functional hepatocytes using published protocols (Huch et al, Nature 2013). These results demonstrate that HepatiCult™ efficiently generates and expands hepatic organoids from mouse livers, and promotes the long-term maintenance and self-renewal of mouse hepatic stem cells that still maintain their differentiation capacity.

W-1188

IPSC-DERIVED HEPATIC ORGANOID RECAPITULATES LIVER DEVELOPMENT AND FUNCTION

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Organogenesis is a complex multicellular process which turns in vitro differentiation and culture of many cells and tissues a difficult task. Primary and iPSC-derived hepatocytes in isolated 2D environment are usually poorly functional and frequently de-differentiate after few days in culture. Many protocols have already demonstrated that co-culture with cellular phenotypes that resembles tissue specific early development enhances final differentiation product and sustain function over time. Organoids have been extensively investigated for modeling early development diseases, tissue engineering approaches, and drug response assays in personalized medicine. Despite great interest, liver organoids have very few available protocols. Here we describe a new protocol that recapitulates early liver development in a 3D environment using donor specific iPSC-differentiated cells encapsulated in matrigel. iPSCs cell lines from human fibroblast and erythroblast were derived and used in these experiments. After pluripotency characterization, every specific donor cell line was further differentiated towards hepatoblast and/or hepatocytes, endothelial cells and mesenchymal cells using standard 2D protocols. The final cellular phenotypes were then mixed in specified proportions and encapsulated in matrigel. Cells survival after encapsulation was on average greater than 90%. After 14 days of encapsulation cells presented spontaneous tissue organization with sustained expression of important markers such as HNF4, ALB, CD31 and CD105. Preliminary experiments shows that secretion of albumin was maintained for several days more than 2D culture of isolated iPSC-derived hepatocytes and primary human hepatocyte. The resulting tissue stained positive for PAS, and presented CFDA-stained bile canaliculi formation. More experiments will be performed to assess the metabolic function of the encapsulated organoids. These preliminary findings supports the importance of multi-cellular 3D culture to enhance liver function in vitro modeling.

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W-1190

EVIDENCE FOR FUNCTIONAL NEURONAL NETWORK FORMATION IN BIOENGINEERED NEURONAL ORGANOIDS

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3D modelling of human brain development in combination with reprogramming technologies is a novel tool to uncover the underlying molecular mechanisms of human neuronal diseases. Despite the advances in human brain modelling there is still little known about functional organization and neuronal network formation. The aim of this study was to develop a protocol for robust and reproducible neuronal differentiation in a defined 3D cell culture system that will allow us to study human neuronal network formation. Human bioengineered neuronal organoids (hBNOs) were formed by culturing pluripotent stem cells (iPSC) in a Matrigel-free collagen matrix for 40-60 days. Neural induction was controlled by SMAD inhibition under defined serum-free conditions. Calcium imaging, transcript and whole mount immunofluorescence (WmIF) analysis were used to screen for optimal protocol development. To obtain insight on the developing cell types and their degree of maturation we performed RNA-sequencing at different time points of hBNO-development (n=3-6/time-point). By culture day 30-40 we observed the highest transcript expression for different neuronal markers providing molecular evidence for the formation of dopaminergic, cholinergic, serotonergic, glutamatergic, and GABAergic neurons. The presence of these neurons was further validated by (WmIF; MAP2, NF, TH, CHAT, VGLUT, GABA,). By day 50-60, astrocyte markers (i.e. GFAP, AQP4, S100β) as well as different collagen types (i.e. COL3A1, COL1A1-2, COL6A1-2) were significantly increased. Calcium imaging revealed neuronal activity by d28 with fast Tetradotoxin (1 µM)-sensitive signals by d40-60. To test neuronal network activity, hBNOs (day 30-60) were subjected to calcium imaging under GABAergic (picrotoxin, 58 µM; saclofen, 330 µM; n=3) or glutamatergic (CNQX, 15 µM; (-) MK-801 25 µM concentration; n=2) inhibition. Interestingly, we observed spontaneously synchronized neurons becoming asynchronous upon GABAR inhibition. Upon washout, the same neurons re-synchronized suggesting the presence of a functional GABAergic network. Taken together, we demonstrate a novel collagen-based, serum-free directed differentiation protocol for the engineering of electrically active neuronal networks from human pluripotent stem cells.

W-1192

HPSC DIFFERENTIATION TO DEFINITIVE ENDODERM CELLS IN DIFFERENT FLEXIBLE THREE-DIMENSIONAL CELL CULTURING SYSTEMS: LIMITATIONS AND POSSIBILITIES

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There is an urgent need for better in vitro liver models for drug testing. Despite development, even in the 21st century there have been withdrawals of new drugs from the markets due to liver toxicity. Cells commonly used for these studies have many drawbacks; primary hepatocytes are difficult to obtain and have a short culturing life, and human hepatocarcinoma cells have abnormalities compared to hepatocytes in vivo. Human pluripotent stem cells (hPSCs) offer a great supply of all kinds of human somatic cells with normal functions. Human PSCs have been differentiated to hepatic-like cells, but obtaining mature hepatocytes has proven challenging. Cells cultured in a three-dimensional (3D) environment have showed to retain better cell polarity and cellular functions than cells cultured on a traditional two-dimensional matrix. Despite this knowledge, a good 3D liver cell model from hPSCs still does not exist; differentiation of hPSCs to hepatocytes is a long stepwise protocol and matrix-based systems used for 3D cell culturing do not allow stepwise tuning of the physical environment and later versatile analysis. The first differentiation step to hepatocytes is definitive endoderm (DE). Due to a great tendency of hPSCs to spontaneous differentiations, culturing environment at this differentiation step has to be very controlled. Here, we compare DE differentiation efficiency of two flexible 3D culturing systems: a matrix-based nanofibrillar cellulose (NFC) hydrogel and a matrix-free suspension culture for hPSC spheroids created in a NFC hydrogel. With both of these systems it is possible to create a whole new environment after every differentiation step. Here we show that based on the expression of DE markers, differentiation efficiency of hPSCs to DE cells is much higher in suspension culture system than in NFC hydrogel. Our simulation data reveal the underlying reason; essential medium supplements: growth factors with a short half-life, restrict the use of matrix-based culturing systems. In conclusion, 3D cell culturing matrices prevent the flow of growth factors. When choosing the 3D cell culturing system, the application and key features in there have to be taken into account.

W-1194

GENERATION OF PERFUSABLE VASCULARIZED HUMAN NEURAL ORGANOIDS USING A COMMERCIAL MINI-BIOREACTOR AND A DEFINED SYNTHETIC EXTRACELLULAR MATRIX

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Pluripotent stem cells (PSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) have enhanced the ability to capture the cellular diversity and native characteristics of human tissues. Using defined methods, PSCs can be differentiated into region-specified neural progenitor cells (NPCs) of the developing nervous system in 2D. However, capturing the cellular diversity, complexity and maturity of the developing human brain is a major challenge in neural stem cell engineering. 3D neural organoids have been generated to address this challenge but to date have limited long term growth potential and suffer from poor reproducibility. Limited growth in neural organoids can be attributed to the lack of a vascular system within the neural organoids resulting in the generation of a necrotic core. Overreliance on Matrigel (a poorly defined material with large batch to batch variation) and lack of control of culture conditions (flow rate) limit their reproducibility. To address this issue, we have generated a vascularized neural organoid using defined differentiation methods, synthetic hydrogels and a custom made bioreactor. PSCs were differentiated into NPCs, endothelial cells, pericytes and microglial precursors for inclusion in our neural assemblies. Vascular networks were formed in 3D in defined synthetic hydrogels under controlled flow conditions in the bioreactor system. NPCs were seeded on the surface of a degradable synthetic hydrogel overlay to form a defined neuroepithelium before integrating with the underlying vasculature. Vascular and neural integration were tracked using a combination of confocal microscopy, label-free fluorescent lifetime imaging microscopy (FLIM), cyrosectioning, and standard florescence microscopy. Thick vascular networks were shown to form within the 3D hydrogels under flow, and endothelial progenitor cells integrated with the developing neuroepithelium. Changes to the vascular network during the integration process were tracked by FLIM. The neuroepithelial layer was allowed to assemble for a further 21 days for additional characterization. Future work will include incorporation of IPSCs from Rett Syndrome patients differentiated into neural, vascular and microglial components for assembly into vascularized neural organoids.

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TISSUE ENGINEERING

W-1198

DEVELOPMENT OF MURIN INCORPORATED SCAFFOLDS FOR FUTURE USE IN TISSUE ENGINEERING AND REGENERATIVE MEDICINE

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Murin is a flavonoid isolated from the leaves of Psidium guajava, being an isomer of quercitin, with a variety of known effects such as, reative oxygen species sequestration, DNA protection, anti-inflammatory, anti-cancer and anti-neurodegenerative properties. Mesenchymal stem cells (MSC) with their capacity of proliferating and differentiating are important and well established tools used in regenerative medicine. Due to the properties of this flavonoid, as well as the MSC capabilities, the aim of this work has been to incorporate murin in polycaprolactone scaffolds produced by the electrospinning (ES) technique and to determine the biocompatibility of them on MSC. To define the dose of murin to be incorporated in the fibers, murin was added to the culture media, with 2,500 cells cultivated in a 96 well plate, in different concentrations, as the following groups: 0, 10, 50, 100, 125, 150, 175, 200, 225 µg/mL of murin. Cellular viability was measured using MTT assay at days 2, 7 and 14. The absorbance results obtained by MTT assay in the groups were, respectively, 0.039±0.015, 0.04±0.006, 0.046±0.009, 0.050±0.010, 0.050±0.012, 0.050±0.010, 0.044±0.009, 0.046±0.020 and 0.036±0.012, for day 2; 0.148±0.058, 0.161±0.071, 0.196±0.078, 0.149±0.061, 0.125±0.062, 0.107±0.058, 0.095±0.051, 0.086±0.045 and 0.060±0.066, for

day 7; and 0.181±0.077, 0.212±0.073, 0.217±0.057, 0.161±0.069, 0.119±0.061, 0.087±0.052, 0.073±0.034, 0.062±0.034 and 0.016±0.020, for day 14 . Based on these results, the dose elected to produce the biomaterial was 100µg/mL as the best range that stimulated cell growth was between 50-100µg/mL. Conversely, higher doses, 200 and 225µg/mL, seemed to present cell toxicity. This biomaterial was analyzed with UV-vis spectrophotometry to quantify the murin incorporation efficiency, as well as the delivery at 0, 3, 6, 9, 12, 24, 48 and 72h using UV-vis spectrophotometry. The incorporation efficiency was 74% and the scaffold delivery was 62% of the total murin incorporated in 72h, with a burst in the first 3h. More analysis is required to better understand and evaluate the effect of murin on the cells; however, as preliminary results, murin seems to stimulate cell proliferation, which would be a good strategy for use in tissue engineering.

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W-1200

RECONSTRUCTING THE TOPOGRAPHICAL REQUIREMENTS OF THE INTESTINAL STEM CELL NICHE USING PATIENT DERIVED CELLS AND DECELLULARIZED SCAFFOLDS

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The intestinal stem cell (ISC) niche, composed of cell and extracellular elements, provides a structured microenvironment that is critical for intestinal homeostasis. While chemical cues for intestinal stem cell behaviour are well described, physical stimuli and topography, also known to impact stem cell behaviour, are less defined. Here, we present our work in mimicking the complex intestinal stem cell niche in vitro, providing insight into the importance of topography in governing states of disease and homeostasis in the gut. Following ethical approval, small intestinal tissue was collected from patients. Crypts were isolated, and organoids cultured from epithelial biopsies of patients from both healthy and disease backgrounds, including intestinal failure. Quantitative real-time PCR analysis of relative mRNA expression markers for intestinal epithelial cells was performed to optimise culture conditions for seeding. The presence of the small molecule Rho-associated protein kinase (ROCK) inhibitor Y-27632 and CHIR99021, a glycogen synthase kinase 3β (GSK3β) inhibitor assisted proliferation of crypt cells, thereby enabling rapid expansion of each patient's cells. In parallel, intestinal fibroblast populations were also isolated for co-culture assays. Whole intestinal tissue specimens were then processed using detergent-enzymatic treatment to derive decellularized intestinal scaffolds from both healthy and disease patient backgrounds. Baseline characteristics of decellularized scaffolds were recorded using histological and electron microscopy analysis. The expanded intestinal epithelial stem cells were then seeded onto decellularized scaffolds derived from either diseased or healthy intestine. Intestinal cell survival, differentiation, morphology and changes in the conformation of the intestinal acellular matrices were subsequently analysed through histology, immunofluorescence, immunohistochemistry and electron microscopy techniques. This work has particular significance for the advancement of intestinal tissue engineering strategies, in the endeavour to provide a realistic alternative to the current available treatments for short bowel syndrome.

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W-1202

ORGANOGENIC RECONSTITUTION OF PARENCHYMAL AND STROMAL TISSUES IN MICE AND PIGS BY DIRECT REPROGRAMMING OF ADULT PROGENITORS WITH A PRENATALLY PIVOTAL GENE AND TARGET PROTEIN

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Whether adult regeneration recapitulates prenatal morphogenesis is frequently debated but rarely studied. Here we report that reprogramming of adult mesenchyme progenitors with Alx3, a prenatally pivotal transcription factor in neural, hair and islet development, not only reverted their fate to prenatal stem cells, but also reconstituted parenchymal and mesenchymal tissues. We further show that this organogenic tissue reconstruction not only ectopically in mice via Wnt and VEGF signaling, but also orthotopically in pigs as a preclinical animal model by delivery of Wnt3a, Alx3's direct target protein. Alx3 expression was exceedingly robust in dental mesenchyme by E16.5 and remarkably reversed to dental epithelium by E18.5 by RNA-Seq, but vanished in the adult. Alx3 gain- or loss-of-functions in E16.5 mesenchyme directly and quantitatively effected dentin formation upon reconstitution with undisturbed

epithelium. ChIP assay showed Alx3 binding to Wnt3a in adult human dental-pulp stem cells, which derive from prenatal dental mesenchyme. Remarkably, Alx3 gain-of-function significantly enhanced Wnt luciferase activity at par with Wnt3a ligand and induced β-catenin transnucleation without appreciable BMP and non-canonical Wnt activation. Alx3 reprograming not only induced vascularized connective tissues with improved cell survival, but also de novo dentin formation via Wnt and VEGF signaling in mice. Alx3 enabled dentin regeneration in adult mice without epithelium signaling, which is prenatally indispensable for mesenchyme differentiation. Alx3 substituted epithelium and orchestrated mesenchyme regeneration by enhancing mesenchymal Wnt3a and β-catenin. In a porcine model following total dental-pulp ablation, Wnt3a-recruited adult endogenous cells not only restored stromal vascular tissues reminiscent of dental pulp, but also odontoblast-like cells extending processes into de novo formed, mineralized tubular dentin in 12 weeks. The newly formed dentin possessed structural and mechanical properties at par quantitatively with the native dentin. PGP9.5+ filaments were present, including myelinated and unmyelinated fibers. Thus, a developmentally pivotal gene, despite its postnatal subsidence, may activate repair signals in adult stem/progenitor cells.

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W-1204

CONTRACTILE IPSC-DERIVED SKELETAL MUSCLE TISSUES FOR MODELING OF HUMAN DISEASE

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Despite established protocols for differentiation of human pluripotent stem cells (hPSCs) into myogenic cells, hPSC-derived skeletal muscle tissues that are able to respond to electrical and biochemical stimuli have not been reported. Here we describe engineering of contractile human skeletal muscle tissues using induced myogenic progenitor cells (iMPCs) generated via a highly efficient differentiation of hPSCs into paraxial-mesoderm followed by transient overexpression of Pax7. Under optimized 3D culture conditions, iMPCs form skeletal muscle tissues (iSKM bundles) containing aligned multi-nucleated myotubes that display functional properties akin to native muscle, including robust calcium transients and positive force-frequency relationship in response to electrical stimulation as well as forceful contractions in response to acetylcholine. When constructed with iMPCs derived from patients with muscular dystrophies, the 3D system allowed us to compare histology, contractile forces and calcium handling between control and disease cells. The iSKM bundles remained functional in culture for at least 1 month and underwent increased structural and molecular maturation, hypertrophy, and force generation. Human PSC-derived muscle bundles hold the promise as a platform for personalized modeling of human muscle disease and screening of candidate therapeutics.

W-1206

DISTAL PNEUMOCYTE DIFFERENTIATION OF BASAL EPITHELIAL STEM CELLS BY INHIBITION OF NOTCH SIGNALING DURING EX VIVO LUNG REGENERATION

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Lung bioengineering on extracellular scaffolds involves combining essential cell populations with corresponding matrices to form living, functional grafts. Epithelial regeneration requires the efficient repopulation of both airway and alveolar cells, along a proximal-to-distal axis. Basal epithelial stem cells (BESCs), identified by p63 and Keratin5 expression, can be routinely isolated from proximal lung tissue and expanded. Yet, a robust source of distal alveolar epithelial cells remains a current challenge to lung regeneration. We report that inhibition of the Notch pathway by targeting gamma-secretase can direct human BESCs toward a distal Type2 pneumocyte fate in vitro. An increase in expression of surfactant protein-C (SP-C, 22.06±0.29-fold increase), ATP binding cassette subfamily A member 3 (ABCA3, 2.79±0.25-fold increase), and Lysosomal-associated membrane protein 1 (LAMP-1, 11.48±0.06-fold increase) was confirmed after 5 days of treatment. Gene expression was confirmed by immunofluorescent cell staining. Surfactant Protein-C production was also measured in conditioned media by ELISA (0.33±1.13 pg/ml untreated vs 1.13±0.09 pg/ml following Notch inhibition). BESCs grown on human acellular lung slices in vitro also increase SP-C expression following 5 days of Notch inhibition (3.03±0.17-fold increase vs. untreated). Additionally, BESCs grown in 3D spheroid culture also up-regulated SP-C following Notch inhibition (5.32±0.89-fold increase). Induction of a distal Type2 pneumocyte fate was confirmed following delivery of BESCs to the airways of acellular rat lung scaffolds and ex vivo biomimetic culture, with delivery of the inhibitors through the vascular perfusate for 5 days (13.08±1.15-fold increase in SP-C). BESCs could also be pre-differentiated in vitro prior to recellularization, and then shown to maintain a distal fate after inhibitor

withdrawal. Analysis of the regenerated lung tissue confirmed extensive alveolar recellularization with organized tissue architecture and morphology. These results demonstrate the potential of endogenous tissue-derived epithelial stem cells for complex ex vivo lung tissue regeneration and contribute to the evolving understanding of epithelial cell identity, hierarchy, and aptitude for repair.

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W-1208

A NOVEL APPROACH FOR RESTORING THE QUANTITY AND QUALITY OF ELDERLY HUMAN MESENCHYMAL STEM CELLS FOR AUTOLOGOUS CELL-BASED THERAPIES

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Degenerative diseases are a major public health concern for the aging population and mesenchymal stem cells (MSCs) have great potential for treating many of these diseases. During aging, the quantity and quality of MSCs declines, limiting their therapeutic potential. Recently, we identified a small sub-population of MSCs from elderly donors that have a "youthful" phenotype. In this study, we isolate four sub-populations of elderly MSCs, based on size and expression of stage-specific embryonic antigen-4 (SSEA-4) using flow cytometry, and compare their characteristics with unsorted populations from young and elderly donors. The results show the overall quantity of MSCs from elderly donors is lower relative to young donors and overall quality is lower based on decreased colony formation capacity and differentiation efficiency. Nevertheless, we were able to isolate a sub-population of small/SSEA-4 positive MSCs from elderly donors that have a youthful phenotype. When this population was expanded on a "young microenvironment" that promotes the maintenance of stemness, their capacity for proliferation and differentiation was maintained. These cells comprise approximately 5-10% of the total MSC population from elderly donors, and studies examining the effect of elderly MSC conditioned media on young cells suggest that the small size/SSEA-4 expressing cells are inhibited by factors secreted by senescent (or pre-senescent) cells. These results suggest the feasibility of establishing personal (autologous) stem cell banks with large quantities of high quality MSCs from elderly donors for treating age-related diseases with autologous cells.

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W-1210

SPHEROIDAL FORMATION PRESERVES HUMAN STEM CELLS FOR PROLONGED TIME UNDER AMBIENT CONDITIONS FOR FACILE STORAGE AND TRANSPORTATION

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Human stem cells are vulnerable to unfavorable conditions, and their transportation relies on costly and inconvenient cryopreservation. We report here that human mesenchymal stem cells (MSC) in spheroids survived ambient conditions (AC) many days longer than in monolayer. Under AC, the viability of MSC in spheroids remained >90% even after seven days, whereas MSC in monolayer mostly died shortly after. AC-exposed MSC spheroids, after recovery under normal monolayer culture conditions with controlled carbon dioxide and humidity contents, resumed typical morphology and proliferation, and retained differentiating and immunosuppressive capabilities. RNA-sequencing and other assays demonstrate that reduced cell metabolism and proliferation correlates to enhanced survival of AC-exposed MSC in spheroids versus monolayer. Moreover, AC-exposed MSC, when injected as either single cells or spheroids, retained therapeutic effects in vivo using mouse models. Spheroidal formation also prolonged survival and sustained pluripotency of human embryonic stem cells kept under AC. Therefore, this work offers an alternative and relatively simple method termed spheropreservation versus the conventional method cryopreservation. It shall remarkably simplify long-distance transportation of stem cells of these and probably also other types within temperature-mild areas, and facilitate therapeutic application of MSC as spheroids without further processing.

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W-1212

TRANSGENIC EXPRESSION OF EPHRINB2 IN STEM CELLS FROM APICAL PAPILLA PROMOTES ANGIOGENIC CAPABILITY OF ENDOTHETICAL CELLS VIA VEGF OVEREXPRESSION

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The success of dental pulp regeneration relies on new blood vessels formation of transplanted tissue and connection with host vasculature. A new promising approach is modulating angiogenesis related molecules, which include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and ephrinB2. Our study aimed to investigate whether transgenic expression of ephrinB2 in stem cells from apical papilla (SCAP) promotes angiogenesis of endothelial cells. Briefly, SCAPs were harvested from human immature wisdom teeth, and identified using CD45, CD105, CD73, CD90 and STRO-1 antibodies. After transduction, both ephrinB2-SCAP and GFP-SCAP showed adequate green fluorescence. Then, they were sorted using Puromycin. Real-time PCR and western blot assay showed that SCAPs transfected with ephrinB2 increase expression of ephrinB2 m-RNA and protein. Furthermore, ephrinB2/GFP-SCAPs were cocultured with human umbilical vein endothelial cells (HUVEC) on Matrigel under normoxia and hypoxia. The results showed that ephrinB2-SCAPs group formed more vessel-like structures, especially under hypoxia. In addition, Real-time PCR and ELISA assay showed that eprhinB2-SCAP group secrets more VEGF than GFP-SCAP group. All of these conclude that SCAP transfected with ephrinB2 promotes angiogenesis of endothelial cells via VEGF overexpression.

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

W-1218

INCREASING MEDICAL STUDENT AWARENESS OF THE ISSCR GUIDELINES FOR STEM CELL RESEARCH AND CLINICAL TRANSLATION, AND ITS RELEVANCE IN THE MEDICAL CURRICULUM

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Recent advances in Stem cell biology impact core subjects of the undergraduate medical curriculum, including embryogenesis, neurosciences, hematology and oncology, with clinical trials using embryonic, adult and induced pluripotent stem cells ongoing in the clinical disciplines of cardiology, neurology and ophthalmology. At the same time, the International Society for Stem Cell Research (ISSCR) cautions that the misuse of stem cells raises both ethical and safety issues impacting patient care. In 2016, the ISSCR published updated Guidelines for Stem Cell Research and Clinical Translation (ISSCR Guidelines), emphasising public communication, in response to concerns regarding public perception of stem cells. As medical students represent an important stakeholder group, it is timely to consider ways that these Guidelines may also be communicated within the medical school framework. The National Institutes of Health (NIH) lists twenty-one stem cell research programs in collaboration with US Medical schools, where opportunities exist for undergraduates to participate in research projects. The ISSCR Guidelines, which reinforces the rigors of scientific research and the ethical issues surrounding stem cells in medicine, provides a useful reference to the junior investigators. This paper reviews the websites of the twenty-one NIH listed stem cell research programs at Universities and Institutions, as well as the top 50 medical schools for research (US News Rankings 2016), as to whether reference to the ISSCR guidelines exists. The study shows that while all the NIH-listed stem cell research programs provide information on stem cells, just four out of twenty-one (4/21) refer to the ISSCR website. Of the top 50 medical schools for research (US News Rankings 2016), sixteen out of the fifty Institutions (16/50), either directly, through hyperlinks, or internal searches within the respective websites, refer to the ISSCR guidelines. In summary, there is scope for further work to promote the ISSCR Guidelines in Medical Institutions with stem cell research programs. This could be achieved in the first instance, by incorporating hyperlinks to the ISSCR Guidelines within the respective websites, such as in the sections “useful links”, “FAQs” on stem cell research, “featured publications” and “research ethics”.

Funding Source: NIL

W-1220

THE FUTURE FOR STEM CELL SCIENCE & REGENERATIVE MEDICINE: FUNDING SOURCES AND INTERNATIONAL COLLABORATIONS

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Recent events in the legislative and executive branches of the U.S. government may become of significant

moment to stem cell science and regenerative medicine in the last part of the 2010s and beyond. These events include the signing into law in December 2016 of the 21st Century Cures Act (21CAA) and the transition in January 2017 in the Office of the U.S. President. The 21st Century Cures Act, for example, contains provisions intended to spur the development of regenerative advanced therapies for serious and life-threatening diseases through application of the U.S. Food and Drug Administration's expedited approval pathways, and for that purpose contemplates the promulgation and adoption of applicable regulations, guidances, and standards by government agencies beyond FDA. At the same time, the new administration is seeking to manage the growth of regulations within the executive branch and also adopt policies that could affect the cross-border movement of scientists, engineers, patients, research participants, and the operations of biopharmaceutical and life science companies with respect to product discovery and preclinical and clinical development. Three related questions arise from these events: (1) what impact will these events have on U.S. government funding of stem cell science and regenerative medicine? (2) what impact would any such changes in U.S. government funding and border control policies have on existing and future international collaboration within stem cell science and regenerative medicine? and (3) how should scientists, engineers, patient advocates, university and company officers, counsel, and others engaged in the scientific, medical, ethical, legal, and social aspects of stem cell science and regenerative medicine channel and coordinate their efforts in light of the answers to the first two questions. We will explore these questions.

W-1222

ENGAGING UNDERGRADUATE STUDENTS FROM VARIED BACKGROUNDS IN STEM CELL RESEARCH

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The educational literature is clear that hands-on activities promote greater learning and understanding of material. This holds especially true for science, as the best way for students to learn science is to do science. The Harvard Stem Cell Institute Internship Program (HIP) began in 2005. Its purpose is to provide an immersive ten-week summer research experience to undergraduate students worldwide. From 2005 to 2015, HIP trained 396 students from 128 different universities and 16 different countries. We evaluated the program in its eleventh year by surveying alumni, yielding a 25.5% response rate. Here we report the efficacy of the program, as well as its overall structure. Given its success, this structure can be mimicked for other summer research programs.

CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

W-1224

OPTIMIZING THE TRANSPORT, STORAGE AND ATTACHMENT CONDITIONS OF CGMP-GRADE HUMAN UMBILICAL CORD MESENCHYMAL STROMAL CELLS (HUC-MSCS) FOR TRANSPLANTATION (HUC-HEART TRIAL)

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The HUC-HEART trial (ClinicalTrials.gov Identifier: NCT02323477) is a phase 1/2, controlled, multicenter, randomized study of the intramyocardial delivery of allogeneic HUC-MSCs in chronic ischemic cardiomyopathy patients. In this ongoing trial, 2x107 cells are administered to peri-infarcted myocardium in patients (n=39) during CABG surgery. Prior to the onset of the cell transplantation, a series of experiments were carried out to optimize the transport and storage conditions for obtaining the highest cell viability, proliferation rate and MSC marker expression of cGMP-grade HUC-MSCs. Cellular actin assembly, as a marker for cell attachment capability were also evaluated in consecutive days of storage prior to transplantation. Four noncommercial transport media supplemented human serum albumin (HSA), and hydroxyethyl starch (HES) [(PBS+1% HSA, 10% HES); (PBS+1% HSA); (Ringer's lactate+1% HSA+10% HES); (Ringer's lactate+1% HSA)] were tested in two transport temperatures (4°C or 22-24°C). The effect of transport media and temperatures was assessed by cell viability and proliferation assays over 6 consecutive days in culture. The highest viability and proliferation rates were obtained, though insignificant, using PBS+1% HSA media at 4°C (viability rate 90.1%; proliferation rate 4.2% fold increase). Time spent for transport was simulated as 6, 12, 24, 36 hours following cell release from the cGMP facility. In order to estimate the cell storage time limit prior to cell transplantation, cell viability and MSC marker

expression levels were evaluated. Increasing storage time intervals did not display a significant decline in cell viability rate or MSC marker expression levels. In vitro total actin signal intensity per cell was examined using in-cell western® assays in two oxygen concentrations (normoxy-21%; hypoxia-5%) in culture days 3, 5 and 7. No significant difference was noted between either days or oxygen concentrations. Overall results suggest that noncommercial, simple transport media formulations with extended storage intervals in ambient temperatures are capable to retain the characteristics of HUC-MSC. The above findings led us to start the HUC-HEART Trial on April 2015; a total of 20 patients received HUC-MSCs; no adverse effect was noted related to cell processing and transplantation.

Funding Source: TUBITAK-#0741-STZ-2014

W-1226

BUILDING THE SANFORD STEM CELL CLINICAL CENTER: HOW CAN AN ACADEMIC MEDICAL CENTER ACCELERATE PHASE I STEM CELL THERAPY CLINICAL TRIALS

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Collaboration is often mentioned as key to successful translation of scientific discovery to clinical trials. But the mechanics of collaboration are often unknown and academics rely on personal drive and serendipity to move forward productive collaborations. Here, we report on how the Sanford Stem Cell Clinical Center accelerated and implemented Phase I stem cell therapy clinical trials at a university medical center. To determine what practices lead to successful translation of stem cell projects to clinical trials, we examine the catalysts for Phase I testing of two cell therapy products at UC San Diego, and accelerators of their implementation. We compare the goal of "design-to-delivery" at the Sanford Center to collaborations in the UCSD Stem Cell Program and the Sanford Consortium for Regenerative Medicine. Design-to-delivery was founded on collaboration in basic science, experience in interinstitutional collaboration, reliance on longstanding preexisting relationships with industry, and priming the pump financially and managerially. The academic setting allowed completion of some "Valley of Death," often-risky activities such as preparation of complete and acceptable IRB submissions, contingency planning, and IND writing. Initiating the stem cell clinical trials required strategic partnerships with industry, philanthropy, the academy, and the state. The key elements were 1. clear and decisive leadership. 2. Excellent stewardship, data analysis, administrative management, and accurate reporting. 3. Proximity to industry partners who were close not only geographically but in relationships over time. 4. A strong mission-drive to accelerate stem cell clinical trials. 5. A focus on sustainability and investment of energy and resources to develop pipeline trials with investigators and with industry partners in pre-IND periods. Clarity of mission, inclusive decision making, strong leadership, adequate staffing and institutional support resulted in collaborations that bound governmental, academic, philanthropic, and industry resources in focused, flexible, and productive projects. Proximity can result in serendipitous innovation. Yet to benefit from spontaneity, academic institutions must plan well in advance to develop the potential to sustain stem cell clinical trials.

Funding Source: Work reported here was made possible in part by grants to University of California, San Diego, to Sanford Consortium for Regenerative Medicine, and to private companies by the California Institute for Regenerative Medicine (CIRM).

W-1228

LARGE SCALE PRODUCTION OF CGMP NSC BANKS FOR USE IN CLNICAL STUDIES TARGETING BRAIN TUMORS

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As cellular therapies continue to advance in promising clinical trials, the demand for reproducible and reliable cGMP manufacturing processes of cell products increases. Large-scale clinical cell banks need to be produced in an efficient culture system with a high cell yield in order to produce sufficient final doses that can be used as an outpatient infusion product. We have previously produced, under GMP conditions, one gene modified neural stem cell (NSC) master cell bank (MCB) and three working cell banks (WCBs) containing up to 9.8E9 cells per bank. These cell banks were used in early phase first-in-human trials for glioma treatment. The adherent cells were cultured in Corning CellSTACKs using GMP compliant reagents and materials. Each bank exhibited similar growth characteristics and final release testing results, including expected levels of transgene expression and normal karyotypic profiles. However, as clinical studies progress, the demand has risen for a manufacturing process with greater yield in less time. Therefore, a new process was developed

to culture the NSCs in the hollow fiber Quantum Cell Expansion System. Our results show that one bioreactor can generate approximately 2.5E9 cells in 10 days while requiring minimal manipulation. To produce the same number of NSCs in CellSTACKs cultures, 10 ten-layer CellSTACKs and 25 days would be required. Our analytical testing show that cells produced in both CellSTACKs and the Quantum Cell Expansion System have the same characteristics and release testing results. Though the two processes vary in cost, time and labor requirements, both methods have proven to be successful in generating high quality cGMP banks for clinical use.

W-1230

HUMAN EMBRYONIC STEM CELL DERIVED THERAPY FOR STARGARDT'S DISEASE

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According to WHO, the number of individuals who lose their vision due to Retinal Degeneration is expected to reach 6 million in 2020. Retinal degenerative diseases affect the retinal region responsible for both the central and detailed vision, the macula, with degeneration begining upon loss of Retinal Pigmented Epithelium (RPE) due formation of drusen (atrophic) or abnormal vessels (exudative). Most of macular degeneration appears in the elderly (AMD), however, hereditary diseases, such as the Stargardt disease, also affect young people.. Since their discovery, human embryonic stem cells (hESC) have been considered a valuable tool for therapeutic purposes. There is evidence showing that transplantation of RPE differentiated from hESCs (hESC-RPE) can recover the photoreceptor and prevent vision loss. Therefore, we aim to develop a safe and efficient treatment strategy for Stargardt's disease based on surgical implantation of hESC-RPE. hESCs were cultured in mTeSR1 medium and spontaneously differentiated in RPE cells under cGMP conditions. These hESC-RPE cells were maintained in serum-free medium (ex-vivo) and manually enriched. Six patients with Stargardt's disease received a hESC-RPE suspension (1.10e6 cell/0.1mL) implanted into the sub-retinal space. The surgery consisted of phacoemulsification plus IOL insertion, pars plana vitrectomy, induction of a limited retinal detachment, cell suspension injection into the subretinal space and air fluid exchange. Oral metilprednisolone and cyclosporine were administered from day 1 tthrough the 3rd month. The patients were evaluated pre- and post-operatively by a complete ophthalmological exam and ancillary examination, comprising imaging and electrophysiological tests. All patients reported improvement in vision. The visual acuity, visual field (Goldmann) and electrophysiological tests showed improvement in all six patients. No adverse effects or surgery-related complications occurred during the entire follow up. Our results suggest that hESCs could be systematically differentiated into RPE and serve as a cell source for several retinal degenerative diseases. The surgical procedure proved feasible and safe, without migration, signs of rejection or inflammation or development of ocular or systemic tumors in the six months follow up.

Funding Source: CNPq

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

W-2002

EXPRESSION OF THE MOUSE OCT4-EGFP TRANSGENE IN PORCINE GERM CELLS AND PREIMPLANTATION EMBRYOS

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Oct4 is an important pluripotency marker in the mouse and humans. It is expressed during preimplantation development; after gastrulation expression is restricted to germ cells. Limited research has been performed on OCT4 expression in the domestic pig that is a valuable large animal model in biomedicine. Previously, we generated Oct4-EGFP reporter pigs carrying the genomic sequence of the murine Oct4 gene fused to the EGFP cDNA (Nowak-Imialek et al. 2011). This animal model was used to analyse the expression profile of the Oct4-EGFP transgene in germ cells and in vivo derived porcine embryos. Here, we report the expression profile of the Oct4-EGFP transgene in oocytes and ovaries from adult sows using flow cytometry and confocal microscope. EGFP expression was not detected in ovarian cells (n=5). Additionally, immature oocytes (n=20) did not show EGFP fluorescence. After insemination of wild-type sows with sperm from Oct4-EGFP transgenic boars, embryos were recovered on Days 3, 4, 5, and 8, respectively, by flushing oviducts and uterus. The EGFP-fluorescence was detected in 8-cell-, 8-16-cell-embryos, morulae and blastocysts from days 5 and 8 using confocal microscope. Embryos up to the 4-cell stage did not show Oct4-EGFP transgene expression. For the first time, EGFP-fluorescence was detected at the 8-cell stage. A strong EGFP signal was observed in morula stages. Similar to bovine and monkey, fluorescence was not restricted to the ICM but

was also seen in the TF of early blastocysts. However, the signal was diminished in TF in blastocysts day 8, whereas expression remained stable in the ICM. These data reveal that the murine Oct4-EGFP transgene used for the production of transgenic pigs became obvious at the 8-cell stage which is consistent with major embryonic genome activation, which initiates in pig at 4-cell stage. Oct4-EGFP expression was downregulated in the porcine TF during blastocyst development and was no more detected at day 8, indicating that OCT4 could serve as useful marker for pluripotency in porcine preimplantation development. In ongoing experiments, we are going to analyse the expression of the OCT4 protein in porcine oocytes and preimplantation embryos to determine whether the murine Oct4-EGFP transgene faithfully reproduces endogenous OCT4 expression in the pig.

PLURIPOTENCY

W-2006

POTASSIUM AS A PLURIPOTENCY-ASSOCIATED ELEMENT IDENTIFIED THROUGH INORGANIC ELEMENT PROFILING IN HUMAN PLURIPOTENT STEM CELLS

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Despite their well-known function in maintaining normal cell physiology, how inorganic elements are relevant to cellular pluripotency and differentiation in human pluripotent stem cells (hPSCs) has yet to be systematically explored. Using total reflection X-ray fluorescence (TXRF) spectrometry and inductively coupled plasma mass spectrometry (ICP-MS), we analyzed the inorganic components of human cells with isogenic backgrounds in distinct states of cellular pluripotency. The elemental profiles revealed that the potassium content of human cells significantly differs when their cellular pluripotency changes. The treatment with two potassium channel blockers, tetraethylammonium and 4-aminopyridine, increased intracellular potassium in human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), accompanied by the dose- and time-dependent downregulation of pluripotency markers. In contrast, treatment with a potassium channel activator diazoxide and a potassium ionophore salinomycin in these cells led to the efflux of intracellular potassium and the upregulation of pluripotency markers. Interestingly, salinomycin facilitates the formation of hiPSCs from cell reprogramming, likely through propelling cell cycle progression and the modulation of endogenous POU5F1 and SOX2 expression. Global gene expression profiling revealed that many genes that are involved in the regulation of cell proliferation, cell growth, differentiation, development of specific cell lineages were differentially expressed at the transcriptional level in hESCs with the modulation of their potassium contents. In addition, cytotoxicity can be selectively induced by the potassium channel blocker 4-aminopyridine in hPSCs rather than their isogenic non-pluripotent cells. Collectively, we report that potassium is a pluripotency-associated inorganic element in human cells and provide novel insights into the manipulation of cellular pluripotency by regulating intracellular potassium.

W-2008

INDUCED PLURIPOTENT STEM CELLS: RESPONSE TO LOW OXYGEN TENSION CULTURE

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Induced pluripotent stem cells (iPSCs) are being widely investigated as a source of cells for regenerative medicine, but methods are needed to assess their quality during the reprogramming and extensive differentiation procedures required to make cellular products. Mitochondria, a critical participant in cell function, can respond to developmental signals and are vulnerable to environmental changes like external toxins and amounts or types of carbon sources; thus, the state of mitochondria can serve as a predictive indicator of cellular history and future behavior. We propose to examine mitochondrial characteristics in iPSCs to assess if they are predictive of cell quality. Specifically, we are interested in one of the most common variability in stem cells' culture which is the level of oxygen during cell culture. Oxygen is an important component of the cellular microenvironment, serving as metabolic substrate and signaling molecule. As reported in literature, reprogramming somatic cells in low oxygen tension improved the reprogramming efficiency and potentially decreased any reprogramming-associated mutation. However, the effect of oxygen level on stem cell self-renewal and differentiation is incompletely understood. We sought to explore the effect of low oxygen tension on iPSCs with the emphasis on assessing if there are correlations between mitochondrial characteristics and performance of iPSCs. We found that there is an increase in mitochondrial mass when iPSCs are grown under low oxygen tension. This mass increase is accompanied by an increase in mtDNA copy number and the level

of proteins of its replication machinery. Surprisingly, we do not observe an increase in the level of oxidative phosphorylation or the level of respiration. As a follow up study, we are using microarray to systematically identify differential gene expression in iPSCs grown under low oxygen tension with the goal of identifying the cause of increased mitochondrial mass. Any results will be validated using gene knock-down experiments.

Funding Source: FDA Intermural Funding

W-2010

INHIBITION OF MEDIATOR KINASE DICTATES THE TRANSCRIPTIONAL LANDSCAPE OF MOUSE AND HUMAN GROUND STATE PLURIPOTENCY

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By enrichment at the correct set of enhancers, the Mediator complex selectively guides RNA Pol II recruitment to orchestrate the transcriptional engine of cell identity. Here, we manipulate Mediator-recruitment of RNA Pol II to characterize the importance of this fundamental axis during cell identity changes, in particular, between undifferentiated pluripotency and early establishment of differentiation. We use multiple genetic and small molecule approaches to show that selective inhibition of the Mediator-kinase module is sufficient to phenocopy the transcriptional landscape of Embryonic Stem cells in the 2i-induced naïve Ground state, with regard to global RNA Pol II loading, transcriptome and proteome. We extend these findings to human stem cells, and observe that in vivo, Mediator-kinase down-regulation coincides with emergence of E4.5 epiblast identity, the correlate of 2i-induced ES cell Ground state pluripotency. In summary, manipulation of Mediator through its kinase module permits selective control of a cell identity transition. In ES cells, this favors pluripotency in a similar manner to 2i, revealing a causal role for Mediator kinase repression in the transcriptional element of Ground state pluripotency. More generally, selective manipulation of the Mediator kinases CDK8 and CDK19 may provide a common approach for the modulation of other cell identity transitions.

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W-2012

COMPARATIVE ANALYSIS OF MITOCHONDRIAL DNA VARIANTS IN HIPSCS AND HESCS THROUGH MASSIVE PARALLEL SEQUENCING

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Human iPSCs and ESCs share numerous features, which include not only their capacity for indefinite proliferation and pluripotency, but also their proneness to genome instability. In this work, we chose to study the mitochondrial DNA (mtDNA) of human pluripotent stem cells, which has, up to now, been subject of very little research. We analyzed four hESCs lines at three different time points (early, mid and late passage) and hiPSCs derived from three different individuals using different reprogramming methods. In total, we have studied 20 hiPSCs lines, some of which clonal lines from the same individual, and evaluated them at two different passages. The analysis of the full mtDNA sequence was performed by ultra deep massive parallel sequencing, calling only single nucleotide variants (SNVs) with heteroplasmic loads >1.5%. The results show that the total number of variants retrieved in the hESC and hiPSC is similar, but that the distribution of the variants in the mtDNA is significantly different. While 52% of the changes fall in coding regions in hESC, in hiPSCs this accounts for 94%. In line with this, the incidence of potentially pathogenic changes was 2.5-fold higher in hiPSC as compared to hESC. Furthermore, these potentially pathogenic variants appeared with higher loads in the hiPSC than in hESC. Interestingly, hiPSC lines derived from the same individual displayed remarkable variation, likely reflecting somatic cellular diversity. The study of the SNVs during long-term culture (over 150 passages) showed no clear indication for a common pattern of increase or reduction in frequency. Some variants exhibited an increase in frequency up to the homoplasmic state at the later passages, while other cell lines lost some variant during culture. We speculate that a number of these variants change in frequency as passenger mutations to chromosomal abnormalities conferring selective advantage. Finally, we did not identify an obvious effect of the different reprogramming methods on the mtDNA mutation load of the resulting hiPSC.

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W-2014

BAF53A IS INVOLVED IN PROLIFERATION OF MOUSE ES CELLS BY REGULATING P53-P21 PATHWAY AND BAF53B COMPENSATES FOR BAF53A FUNCTION

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The mammalian SWI/SNF chromatin-remodeling complex, also called BAF (Brg1/Brm associated factor) complex, is involved in a variety of cellular processes, including differentiation, proliferation and oncogenesis. The pluripotency and self-renewal capacity are two major characteristics of embryonic stem (ES) cells; and ES cell-specific BAF (esBAF) complex is known to regulate these characteristics. Baf53a is one of the components of esBAF complex, and here we examined molecular functions of Baf53a in mouse ES cells. Baf53a was expressed in undifferentiated ES cells and its expression decreased after differentiation in ZHBTc4 ES cells (Oct3/4 conditional knockout ES cells). Tetracycline-inducible Baf53a conditional knockout (KO) ES cells exhibited that self-renewal marker genes, including Nanog, Oct3/4, Dax1, and Esrrb, were expressed in Baf53a KO ES cells. Direct cell counts and WST-1 assay revealed that proliferation of Baf53a KO ES cells was completely repressed; and p53, p21 and cleaved caspase 3 were induced after Baf53a depletion. Colony formation assay revealed that ES cell colonies were disappeared after Baf53a depletion; however, colony formations were recovered by an artificial expression of Baf53a, but not Baf53a M3 mutant (E388A/R389A/R390A, a dominant lethal mutant form in yeast) in Baf53a KO ES cells. Interestingly, forced expression of Baf53b, a homologue of Baf53a, also induced colony formation of Baf53a KO ES cells. Furthermore, alkaline phosphatase activity, which is the marker for undifferentiated state of ES cells, was detected in Baf53a- or Baf53b-expressing Baf53a KO ES cells; and these ES cells expressed self-renewal marker genes (Nanog, Oct3/4, Dax1, Esrrb, Klf4 and Rex1) and exhibited normal proliferation with repression of p21 expression. Taken together, our findings suggest that Baf53a, one component of esBAF complex, is involved in proliferation of ES cells by regulating p53-p21 pathway; and Baf53b is able to compensate for functions of Baf53a.

W-2016

THE ARYL HYDROCARBON RECEPTOR IS DOWNREGULATED DURING THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO THREE DIFFERENT LINEAGES

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The aryl hydrocarbon receptor (AHR) is a ligand dependent transcription factor previously known for its role in mediating the toxic effects of various environmental contaminants. Recent advances in the field, however, have broadened its role in normal physiologic processes. Moreover, the role of AHR in cells with high developmental potential (e.g. hematopoietic, neural, brest cancer stem cells/progenitors) has been established recently. However the expression and role of AHR in human embryonic stem (hES) cells and its differentiating counterparts has not been described yet. Thus our aim was to characterize the expression profile of AHR and investigate its role in pluripotent hES cells and during the early differentiation. Our experiments show that AHR is expressed in pluripotent hES cells and the known carcinogen and most potent ligand of AHR – TCDD – has no effect on the expression of pluripotency factors as well as the cell cycle. This indicates that AHR is constitutively activated, which was also corroborated by immunofluorescence analysis. Subsequent analysis of AHR expression during differentiation of hES cells into embryoid bodies showed that AHR is downregulated, contrasting previously published data in regard of murine ES cells and differentiation of hES cells with retinoic acid (RA). By induction of differentiation with RA in hES cells, we indeed observed concomitant upregulation of AHR. Interestingly, directed differentiation towards three different lineages, by using two commercially available differentiation kits, showed a robust downregulation of AHR at both mRNA and protein levels. This indicates that AHR is indeed downregulated as specific differentiation of hES cells into three different lineages proceeds. Since the regulatory region of the AHR gene has binding sites for pluripotency factors and vice versa, our currently ongoing experiments are elucidating the role of AHR in pluripotency and differentiation.

W-2018

WNT-DRIVEN G1 LENGTH LINKS SINGLE CELL VARIATION TO DIFFERENTIATION COMPETENCY OF PLURIPOTENT STATES

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The link between single cell variation and population level fate choices lacks a mechanistic explanation despite the extensive observations of gene expression variation among individual cells. Here, we measured absolute lengths of G1 phase at a single cell level and found that single human embryonic stem cells (hESCs) have different and biased differentiation potentials toward either neuroectoderm or mesendoderm depending on their G1 lengths even before the onset of differentiation. Therefore, single cell variation in G1 length establishes a probability distribution that determines the fate of the population. Although sister stem cells generally share G1 lengths, a small and variable proportion of cells do not. This asymmetry in G1 lengths can shift the G1 length probability curve. Environmental levels of WNT control the G1 length distribution curves, which defines a continuum of pluripotent states from the naïve to primed state and their differentiation competencies. Our findings suggest G1 length distribution as a core mechanism linking cell state transition to population outcome.

W-2020

PERIOD 2 CYCLES IN A CIRCADIAN PATTERN IN PREIMPLANTATION MOUSE EMBRYOS AND A FRACTION OF PLURIPOTENT MOUSE EMBRYONIC STEM CELLS

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There is increasing evidence that circadian gene control may be important to embryo development and stem cells. We have reported mRNA expression of core circadian oscillators in 8-Cell human embryos, but not in cultured human pluripotent stem cells. More recently we reported that 52% of 457 growth factors and receptors are circadianly expressed in various mouse tissues. Moreover, 73% of the growth factor family genes over-detected on the 8-cell embryo microarrays are known to be circadianly expressed. Hence, in addition to the canonical growth factor/receptor pathway control of cell division and differentiation, there may be an over-arching circadian control due to the availability of growth factors/receptors at limited times of day. To begin to explore circadian gene expression and growth factoresponses in embryonic stem cells, we have derived mouse embryonic stem cells from a mouse transgenic for Period 2 coupled to luciferase, the "Per2Luc" mouse. We have reported luminometer studies that revealed constant light output by a minimum of 50,000 cells, for 48 hours in the presence of LIF, with light beginning to oscillate at 24 hour oscillations if LIF was removed. Sensitive time-lapse photo microscopy revealed approximately 10% of the stem cells produced detectable light. We have hand annotated 100 individual cells, or small clusters, producing light over a 19 hour period. Each bioluminescent frame capture was accompanied by a bright field frame capture for comparison. Light was detectable from approximately 10% of the small cell clusters, perhaps individual cells, at all times during the 19 hours. Of the 100 annotated light-producing cells, approximately 53% oscillated with periods of brightness from 8 to 16 hours. The remaining glowing cells exhibited variable periods with two or more cycles during the 19 hours. We also hand annotated light production by 5 Per2Luc embryos continuously filmed from the one cell to the blastocyst stage. Each blastomere produced light in a circadian pattern, not always synchronous. The inner cell mass produced significantly more light than trophoblast. These findings suggest Period 2 is expressed in a circadian pattern in at least a fraction of pluripotent stem cells, not in synchrony. This system paves the way to study the effects of individual growth factors on Period 2 expression.

Funding Source: Bedford Research Foundation

W-2022

THE AUTOIMMUNE REGULAR GENE (AIRE) DRIVES THE INDUCTION OF EMBRYONIC DEVELOPMENTAL PLURIPOTENCY

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The autoimmune regulator (Aire) protein plays an essential role in thymic education of immune system and preventing the release of auto-reactive T cell, which mediated autoimmune diseases. Peripheral tissue antigen expression by Aire mediated global gene induction leads to their representation on the cell surface and thus to the identification of auto-reactive T cell and immune-tolerance. Remarkably however, Aire expression is not restricted only to the thymus, but is also expressed during early pre-implantation development, where its function remains to be explored. Further, Aire is conserved throughout vertebrate evolution and is found in all distant vertebrate species including Zebrafish. We have systematically studies the role of Aire in assembling pluripotency in vitro and in vivo. While Aire is dispensable for murine ESC maintenance, induction of Aire during somatic cell reprogramming significantly increases

iPSC formation, and can substitute for exogenous Sox2 induction. In vivo experiments in both mice and zebrafish early development, show a significant decrease in zygote toward blastocyst maturation, 15% and 40%, respectively, upon Aire depletion. Our mechanistic and expression profile studies implicate Aire in co-opting its role as a global transcriptional activator, for boosting transcriptional activation of the genome, zygotic or during iPSC formation, which is a critical event leading to assembling the pluripotency state in vitro and also proper development in vivo. Collectively, we elucidate a previously unidentified cross-specific role for Aire in safeguarding robust early embryonic development and reprogramming.

W-2024

AKT3 IS RESPONSIBLE FOR THE SURVIVAL AND PROLIFERATION OF EMBRYONIC STEM CELLS

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The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB/Akt) pathway plays an important role in regulating cell proliferation, metabolism, and survival. There are three Akt isoforms encoded by different genes, namely Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ, each with conserved peptide sequences from mice to humans. But their roles in pluripotent stem cell maintenance are not fully defined. Here we investigated Akt functions in mouse embryonic stem cells (ESCs). We first inhibited the total Akt activity by treating mouse ESCs with an Akt-specific allosteric inhibitor MK2206 (MK). A dose-dependent inhibition effect on ESC colony growth was observed. Annexin V apoptosis assay using flow cytometer revealed that MK treatment led to a significant increase in both early- and late-stage apoptotic cells. To identify the Akt isoforms responsible in this process, RNA interference (RNAi) was used to deplete Akt isoforms individually or in combination in mouse ESCs. When depleting Akt3 alone, but not blocking Akt1 and Akt2 separately or in combination, obvious apoptosis was observed. In addition, depleting Akt3 alone led to a cell cycle arrest at G1 phase whereas blocking Akt1 and Akt2 did not influence cell cycle. Moreover, LY294002, a PI3K inhibitor that minimizes the phosphorylation and activation of Akt kinase, had similar effects on ESC apoptosis and cell cycle arrest. These effects of LY294002 could be reversed by overexpression of a constitutively active CA-Akt3, but the reversal was aborted when the kinase dead KD-Akt3 with the lysine 177 of the ATP-binding site mutated to methionine was overexpressed. Hence, the regulatory role of Akt3 on ESC survival and proliferation is largely dependent on its kinase activity. However, no significant changes were found for the expression of pluripotency surface maker SSEA-1 or several key pluripotency markers, including Oct4, Sox2, Nanog, and Esrrb upon Akt3 knockdown. Our results revealed an Akt3 isoform-specific mechanism for ESC survival and proliferation.

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W-2026

ENHANCED HUMAN NAÏVE PLURIPOTENCY GROWTH CONDITIONS THAT ENDOW TOLERANCE FOR LOSS OF EPIGENETIC REPRESSORS

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Different conditions have been devised to isolate human MEK/ERK independent naïve pluripotent stem cells (PSCs) that are distinct from conventional/primed PSCs. We have previously published naïve human stem cell medium, designated as NHSM (Gafni et al. 2013). The naïve conditions described thus far endow human PSCs with different extents and variety of naïve features. Now, we report that follow-up dissection of these states indicates that all previously published naïve PSCs fall short from capturing several defining functional and molecular features of murine naïve ESCs, including: (1) ability to maintain pluripotency in the absence of global DNA or mRNA methylation repressive marks or miRNAs (2) ability to maintain naïve pluripotency in the absence of exogenous L-glutamine. We engineered novel reporter systems with CRISPR/Cas technology that allow screening for naïve PSC maintenance in the absence of exogenous L-glutamine or endogenous DNMT1/METTL3/DGCR8 enzymes. Screening assays identified that supplementation of NHSM with a small molecule inhibitor for nuclear WNT signaling and SRC endows human naïve PSCs with the latter unique characteristics. We designated these conditions as Enhanced NHSM (ENHSM) conditions, and systematically characterize newly derived genetically unmodified ESCs and iPSCs lines. ENHSM supports the growth of human and monkey PSCs on feeders

and feeder-free culture with all defining features of mouse naïve ESCs. Importantly, these conditions yield differentiation competent SSEA3+/SSEA4 cells with global DNA hypomethylation, imprinting maintenance and X-chromosome reactivation. Transgene and Feeder-free culture of hPSCs supported by ENHSM allowed us to dissect molecular pathways governing their naïve state. Interestingly, we found that β-CATENIN stabilization enhanced differentiation of ENHSM cultured hPSCs. Moreover, depletion of WNT inhibitor from ENHSM deteriorates the naive characteristics of hPSCs, a phenotype that was rescued by the deletion of β-CATENIN. Importantly, these data suggest opposing roles for WNT signaling pathways in promoting naïve pluripotency between mice and humans. Collectively, we devise human naive PSCs with enhanced molecular and safety features, and provide species-specific insights on the regulation of naïve pluripotency.

W-2028

SETTING HUMAN "GROUND' STATE THROUGH CDK8/19 INHIBITION

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Embryonic Stem Cells (ESCs) can interconvert between two pluripotent states: "ground" and "primed". Dramatic changes in transcription, epigenetics, metabolism and DNA methylation are known to accompany the inter-conversion between these two states. Chemical inhibition of MEK and GSK3b ("2i") can induce "ground" state in a small fraction of human ESCs, and this can be enhanced by concomitant inhibition of p38 and JNK. Here, we report that chemical inhibition of CDK8 efficiently induces the "ground" state in both human and mouse ESCs. Moreover, "ground" state human ESCs can be stably maintained, passaged and amplified by the sole addition of a chemical inhibitor of CDK8/19. CDK8 is part of quaternary complex, formed by MED12/MED13/CCNC/CDK8, which associates to the multiprotein complex known as MEDIATOR. In turn, MEDIATOR-CDK8 has a role as transcriptional co-factor. In summary, we report a novel, efficient, stable and simple method to capture the "ground" state of human ESCs.

Funding Source: This Project is funded by the European Research Council (ERC).

PLURIPOTENT STEM CELL DIFFERENTIATION

W-2032

MULTI-OMIC AND SINGLE CELL TECHNOLOGIES IDENTIFY NOVEL CELL SURFACE MAKERS AND GENE REGULATORY PROCESSES IN CRANIAL NEURAL CREST CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Neural crest cells (NC) emerge at the neural plate border along the rostro-caudal axis during early embryonic development of vertebrates and migrate extensively within the embryo, to give rise to mesodermal and ectodermal cell types. NC behaviour and specification have been mainly studied in mouse and avian models due to the low availability of early human embryos. While human pluripotent stem cells have been successfully used to generate NC cells in vitro, little is known about the molecular mechanisms involved in specification of human cranial or trunk neural crest cells and specific cell surface markers to facilitate the isolation of pure populations of NC cells do not exist. Indeed, we demonstrate that neither p75NTR nor HNK-1, cell surface markers widely used to define and isolate NC cells, specifically mark NC cells that express SOX10, the master transcriptional regulator of this cell type. To address this, we inserted a reporter cassette carrying 2A-linked photo-convertible fluorescent protein mMaple and a puromycin resistance gene into the 3' UTR of the SOX10 locus in hiPSCs using CRISPR/Cas9 genome editing technology. We show that all mMaple-expressing hiPSC-derived NC cells express SOX10 and display resistance to puromycin, thus enabling their facile isolation. Remarkably, SOX10:mMAPLE+ cells can be expanded up to 35 fold in our differentiation medium in the absence of antibiotic selection and without losing their SOX10 expression, suggesting that our defined small molecule differentiation medium is sufficient to maintain the SOX10 expressing NC cells. We find that SOX10:mMaple+ cells express NC markers TFAP2a and FOXD3 and can differentiate into tri-potential MSCs and Schwann cells, but in contrast to p75NTR sorted NC

cells, do not generate peripheral neurons. Bioinformatic analysis of the transcriptome and proteome of the SOX10:mMaple+ NC cells permitted identification of two unique cell surface markers specific for these cells and suggest that the population primarily consists of cranial NC cells for which in vivo migration assays are underway. The SOX10:mMaple+ and negative NC cells were subjected to single cell transcriptomics to delineate the population heterogeneity, transcriptional dynamics and molecular determinants of early cranial human NC cells.

W-2034

TIMELY INDUCTION OF GATA2 PROMOTES HEMATOENDOTHELIAL DEVELOPMENT FROM HUMAN PLURIPOTENT STEM CELLS

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GATA2 is a master regulator of hematopoiesis, highlighted by the fact that Gata2 null mutant mice die at E 10.5 from a complete failure of primitive and definitive hematopoiesis. New findings implicate GATA2 haploinsufficiency with some familial cases of AML/MDS and MonoMac syndrome. However, its specific function during human early hematopoietic development remains unknown. We generated human induced pluripotent stem cells (hiPSCs) in which GATA2 signaling could be timely induced by doxycycline. Overexpression of GATA2 at the point of origin of mesoderm (day 2-4 of EBs differentiation) resulted in the expansion of hemogenic progenitors (HEPs; CD31+CD34+CD43-CD45-; ~5 fold) and promoted their further commitment into hematopoietic progenitors (HPCs: CD34+CD43+CD45+; ~5 fold), without altering proliferation and survival of cells. Functionally, GATA2 enhanced the clonogenic potential of hematopoietic-derived cells (~4 fold); however GATA2 on its own was not sufficient to confer in vivo long-term engraftment potential to hiPSC-hematopoietic derivatives. On the other hand, GATA2ko iPSC lines exhibited significant reduction in the production of HPCs. RNA-sequencing showed that GATA2 induced 109 genes and repressed 385 (twofold, P value < 0.01), in FACs-sorted HEPs at day 10 of EBs development. GATA2-induced genes showed the highest enrichment in GO categories reflecting hematopoiesis and regulation of transcription factors. These included factors implicated in definitive hemogenic endothelium and several HSC regulators (RUNX1, MYB, NFE2, PU.1, GATA1, STAT1, ITGA2B, CSF1R, SPN/CD43, ALDH1A1). Interestingly, another highly enriched category was immune response (IRF7, IFI27, IFIT1, TMEM173, IFI6, IFITM1, TRIM6, TRIM14, TRIM25). On the other hand, endothelial genes (JAG-1, KDR, SOX17) and several cardiac regulators (TBX3, TNNC1, MYOCD, NR2F2, CCML2) were repressed upon GATA2 induction. We also generated iGATA2-iPSCs carrying Brachyury (T)::Red reporter, to mark and sort Red+ mesodermal cells expressing GATA2 during early stages of EBs differentiation. Chip-Seq analysis in day 4 EB T+/GATA2+ cells are now ongoing. Our findings confirm a critical role of GATA2 in human hematopoiesis and suggest that GATA2 regulates hematoendothelial transition during human iPSC-hematopoietic development.

W-2036

A DELAYED H3K27ME3 ACCUMULATION AFTER DNA REPLICATION OF EMBRYONIC STEM CELLS OPENS CHROMATIN FOR LINEAGE SPECIFIC TRANSCRIPTION FACTORS TO BIND AND INITIATE DIFFERENTIATION

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Discovering how to differentiate pluripotent stem cells (embryonic stem cells (ESC) and induced pluripotent stem cells (iPS)) into midbrain dopaminergic (mDA) neurons is both a fundamental biological question and a central issue in developing new therapeutic tools for Parkinson Disease (PD). Differentiation is accompanied by well-characterized massive changes in the epigenome of stem cells. However, the nature of epigenetic marking during DNA replication, and especially how and when this marking undergoes changes during differentiation, is not known. Using single-cell and gene-specific analyses, we discovered that during the first hours of induction of differentiation of mammalian ESCs, accumulation of the repressive histone mark H3K27me3 is delayed after DNA replication, indicative of decondensed chromatin structure in all regions of the replicating genome. This delay provided a critical 'window of opportunity' for recruitment of lineage-specific transcription factors (TFs) to DNA, such as SIP1, Lmx1a and Foxa2. Increasing the levels of post-replicative H3K27me3, or prevention of S phase entry inhibited recruitment of new TFs to DNA and significantly blocked cell differentiation.

Newly acquired knowledge of these issues may prove pivotal in designing new strategies for directing stem cells to exclusively become subtype specific neurons (for example, mDA neurons), with significant outcomes for PD and other human neurodegenerative diseases.

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W-2038

SINGLE CELL ANALYSIS OF TRANSCRIPTION FACTOR EXPRESSION DURING DIFFERENTIATION OF PSC TO CARDIOMYOCYTES USING FLOW CYTOMETRY

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The ability to direct human Pluripotent Stem Cells (hPSCs) towards differentiated cell phenotypes offers great potential for personalized and regenerative medicine. The identification of key transcriptional regulators of pluripotency enable researchers to derive a variety of differentiated cell types with a high degree of control and precision. Quantification of the dynamic expression patterns of transcription factors that underlie cardiomyocyte differentiation often relies on the detection of mRNA transcripts via qRT-PCR in cell and tissue lysates made from heterogeneous populations of cells. While this approach is highly sensitive and can be performed using small amounts of material, it does not provide resolution at the level of individual cells. An alternate approach is to use specific antibodies for detection and quantification of transcription factor expression at the single cell level. Flow Cytometry is ideally suited for this type of analysis. Here, we describe a flow cytometric method for quantification of Oct4, a recognized marker of pluripotency, and Nkx2.5, a marker of cardiac fate, in hPSCs induced to differentiate towards cardiomyocytes. Undifferentiated H9 hPSCs were cultured and expanded, and induced to differentiate into a cardiomyocyte fate. Cells were tracked over a 10 day culture after differentiation was initiated. Cells were labeled simultaneously using fluorescently tagged antibodies to OCT-4 and Nkx2.5 then analyzed by flow cytometry. Prior to differentiation nearly all cells expressed the Oct4pos/Nkx2.5neg phenotype, consistent with a pluripotent state. With induction, expression of Oct4 declines, congruent with a loss of pluripotency, and a transition to a terminally differentiated cardiomyocyte phenotype is seen as expression of Nkx2.5 increases. For research use only.

W-2040

INTERACTIONS BETWEEN OTX2 AND NANOG REGULATE SELF-RENEWAL NETWORK

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Nanog is a transcription factor that plays a central part in the gene regulatory network that maintains and induces pluripotency. The activation of direct Nanog targets, such as Esrrb, is important for mediating Nanog function. To address the roles of targets repressed by Nanog, we focused on Otx2. Otx2 over-expression strongly induces mouse ES cell differentiation and this effect is dominant over the opposite effects exerted by Nanog or Esrrb when co-expressed. Surprisingly, Otx2 can directly interact with Nanog through a mechanism similar to the interaction of Nanog and Sox2. We identified the exact aromatic residues that mediate the interaction between Nanog and Otx2. Mutation of the responsible residues impairs the ability of Otx2 to regulate downstream targets and to induce differentiation.

W-2042

STUDY ON ESTABLISHMENT OF MYOCARDIAL DIFFERENTIATION OF INDUCIBLE PLURIPOTENT STEM CELLS (IPSC)

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Induced pluripotent stem cells (iPSCs) are expected to yield novel therapies with the potential to solve many issues involving incurable diseases, drug development, because iPSCs are free for the ethical and immunological problems that have obstructed the clinical applications of embryonic stem cells. However, as iPSCs research has progressed, new problems have emerged that need to be solved for clinical application of iPSCs. For example, iPSCs have defects on genetic instability caused by integration of reprogramming transgenes and accumulation of mutations during the reprogramming process and cell passaging. Thus, that is required that a comprehensive understanding of characterization and quality control about differentiation from iPSCs. In the present study, we identified that maintenance and genetic stability of undifferentiated iPSCs(hFSiPSC-1) in feeder-free culture medium by Micoplasma test, Karyotype analysis, AP staining, Immunofluorescent staining (Oct4, SOX2, TRA-1-60, SSEA-4). Next, we confirmed the differentiation potential under various conditions to establish myocardial cell differentiation. To determine whether iPSCs was differentiated to cardiomyocyte, we identified by immunostaining

with cardiac differentiation associated markers, such as brachyury, Nkx2.5, Isl1. Moreover we identified beating of differentiated cells that is charateristic of cardiomyocyte. We confirmed that quantity of iPSCs is required more than 2.5x105cells/well for effective cardiac differentiation in 12 well plate, the beating cluster of cells were observed after 15 days. Through further study, we will establish a criterion for evaluating the differentiation ability of induced pluripotent stem cells to differentiate into myocardial cells.

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W-2044

GENERATING FUNCTIONAL KIDNEY CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Chronically damaged nephrons deteriorate progressively towards end stage renal disease (ESRD), owing to the limited regenerative capacity of adult mammalian kidneys. The generation of renal cells and tissues from human pluripotent stem cells (hPSC) is a promising strategy to develop regenerative therapies for ESRD. In this study, we established a protocol to differentiate hPSCs to renal progenitors, capable of producing nephronal cell types and structures in vitro and ex vivo. An effective combination of growth factors obtained after intensive screening, was used to create an 8-day-protocol that steered hPSCs to the renal lineage by a step-wise process outlining the embryonic milestones in kidney organogenesis. Six days after growth factor treatment, a mixture of SIX2+/CITED1+ cells representing metanephric mesenchyme and an HOXB7+/GRHL2+ population indicative of ureteric bud progenitors was obtained that developed into LGR5+/JAG1+/WT1+ renal vesicle cells by the eighth day. Prolonged cultivation of these progenitor cells in three inductive media resulted in generation of WT1+/PODXL+/SYNPO+ podocyte-precursors, PDGFRß+/DESMIN+/αSMA+-mesangial cells and fractions of proximal, distal and collecting duct tubular epithelial cells expressing segment-specific transporters in vitro. Functionality of these cells in terms of sodium and glucose uptake, relocalization of AQP2 in response to hormonal stimuli and albumin endocytosis could be demonstrated.Moreover, when day 8 cells were allowed to differentiate spontaneously, renal organoids developed in culture. Taken together, we demonstrate that starting from hPSC; our in vitro protocol generates a pool of nephrogenic progenitors reminiscent of nascent nephrons that can be further coaxed into specialized nephronal cell types after 14 days. This simple and rapid method to produce renal cells from a common precursor pool in 2D culture provides the basis for scaled-up production of tailored renal cell types, applicable for drug testing or cell-based regenerative therapies.

W-2046

ANALYSIS OF MIRNAS EXPRESSION DURING DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS TO DEFINITIVE ENDODERM

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Pluripotent stem cells hold great promise of treating chronic liver diseases through cell replacement therapy, owing to the ability to self-renew and differentiate into all cell types. However, the current available differentiation protocols have yet to generate fully functional hepatocytes in vitro. Most, if not all of the hepatocyte-like cells derived from stem cells often display high oncofetal marker expression and rapidly lose their metabolic functions. Increasing evidence showed that microRNAs (miRNAs) play a crucial role in controlling the embryonic stem cell (ESC) state. Although there are many reports about their role in maintaining ESC pluripotency, the mechanisms by which they promote cell differentiation remain largely unknown. In the present study, we aim to identify endodermal-inducing miRNAs involved in hepatic differentiation. Here, we induced mouse embryonic stem cells (mESCs) to definitive endoderm and characterized them with endoderm specific markers, such as Sox17 and Foxa2. To investigate miRNAs up- or down-regulated upon endoderm differentiation, we performed miRNA array analysis on mESCs and differentiated endodermal cells. By validation through qPCR, we successfully uncovered the miRNAs that are differentially expressed during the course of endoderm differentiation. We further evaluated the functional roles of the selected miRNAs by transfecting miRNA mimics or inhibitors into undifferentiated mESCs and examined their impact on the induction of endodermal differentiation markers. In addition, we also studied the mechanism by which these miRNAs regulate endoderm differentiation through identification of their targets. Collectively, our findings revealed a miRNA-mediated mechanism that regulate endoderm differentiation from mESCs, showing their importance in mESC lineage commitment.

W-2048

FUNCTIONAL EVALUATION OF MESENCHYMAL STEM CELLS DERIVED FROM CANINE INDUCED PLURIPOTENT STEM CELLS

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Mesenchymal stem cells (MSCs) exhibit broad immune modulatory activity in vivo and can suppress T cell and dendritic cell activation in vitro. Currently, MSC for clinical usage are derived from adipose or bone marrow, utilizing allogeneic MSC due to the superior immune modulatory activity of MSC from young donors. However, use of allogeneic MSC from multiple unrelated donors makes it difficult to standardize MSC cellular products with uniform immune modulatory properties for clinical trials, in both veterinary and human patients. One solution to these problems is the use of MSC derived from induced pluripotent stem cells (iPSC), as iPSC-derived MSC have nearly unlimited proliferative potential and exhibit in vitro stability with respect to surface phenotype. Therefore, we evaluated functional properties of canine iPSC-derived MSC, including their immune modulatory properties and their potential for teratoma formation. Canine iPSC-derived MSC were generated by culture of iPSC; iPSC-derived MSC (iMSC) downregulated expression of pluripotency genes and appeared morphologically similar to conventional MSC. Importantly, the iPSC-derived MSC (iMSC) retained a stable phenotype even after multiple passages, did not form teratomas when inoculated in immune deficient mice, and did not induce tumor formation in purpose-bred dogs following systemic injection. The potency of iMSC was similar to that of adipose-and bone-marrow derived MSC with respect to suppression of T cell and DC activation. We concluded therefore that iPSC-derived MSC were phenotypically stable, immunologically potent, and unlikely to induce tumor formation, and therefore represent an important new source of cells for therapeutic modulation of inflammatory disorders.

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W-2050

IN VITRO CHARACTERIZATION OF MIRNA-MEDIATED CONTROL OF SATB2 EXPRESSION DURING CORTICOGENESIS

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Although the key genes responsible for the generation of the different pyramidal neurons composing the six cortical layers are known, the mechanisms accounting for their expression in specific progenitor cells and at different times of cortical neurogenesis is not completely understood. Satb2 is a crucial transcription factor of cortical layering, as its activation specifies upper neuron identity, inhibits the transcription of the deep neuron identity Ctip2 gene and establishes the end of corticogenesis. In mice, the onset of Satb2 protein detection (E15.5) is two days later that the onset of its mRNA detection (E13.5), suggesting that post-transcriptional mechanisms could account for the time of Satb2 activation. We assayed the involvement of miRNA-mediated RNA silencing of Satb2 in an in vitro system of corticogenesis, using neuralized mouse ESCs. Si-RNA- mediated DICER knock-down at early stages of in vitro ESC neuralization (at day in vitro 5-6, DIV5-6), but not at later stages (DIV7-7), induced significant increase of Satb2 translation in post-mitotic cells at DIV 14. Moreover, Satb2 3'UTR inhibited GFP translation from a reporter vector when it was transfected at early (DIV12) but not at late (DIV 17) stages of in vitro corticogenesis. We looked for candidate miRNAs that could be involved in targeting Satb2. Among the 100 most expressed miRNAs in cortical progenitor cells sorted at DIV 10, 12, 16, 20 or 26, 13 and 12 showed decreasing and increasing expression over the in vitro process of corticogenesis, respectively. While 9 out of the 13 decreasing are predicted to bind to Satb2 3'UTR, none of the 12 increasing show any predicted binding site. Functional experiments of validation of the 9 miRNAs expected to inhibit Satb2 translation are in progress. Our observations suggest that the activation of Satb2, and thus the control of the completion of the corticogenetic process, might considerably be miRNA-dependent.

W-2052

TARGETING THE HYPNOZOITES: TOWARDS AN IN VITRO SYSTEM FOR ANTIMALARIAL DRUG DISCOVERY

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The majority of the available antimalarials on the market exclusively target blood stages of malaria parasites. However, in order to eliminate this infectious disease, we need drugs that prevent parasite transmission and eliminate the dormant liver stages found after infection with Plasmodium vivax and Plasmodium ovale. These quiescent forms called hypnozoites, can survive for months in the liver and reactivate into mature liver schizonts causing relapses in patients. Up to date, Primaquine is the only available and efficient drug against the dormant hepatic forms but its use is limited due to severe side effects and its lack of activity against blood stages. Our aim is to develop an in vitro model for the malaria liver stages that allows medium throughput screening of compounds for efficacy against hypnozoites. As P. vivax only infect reticulocytes, in vitro investigations are hardly feasible. Therefore, the primate malaria P. cynomolgi parasite was chosen. To be species compatible and to have an option for scaling up the hepatocytes, we decided to generate hepatocyte-like cells using induced pluripotent stem cells (iPS) derived from cynomolgus monkey somatic cells. IPS cells were first established by reprogramming of cynomolgus monkey fibroblasts and T-lymphocytes using the four Yamanaka transcription factors Oct4, Sox2, Klf4 and, c-Myc. The pluripotency of these primate cells was assessed via immunofluorescence staining and flow cytometry. The produced iPS cells were then differentiated into hepatic cells via a step-wise approach which recapitulates essential stages of liver development. Characterization of the resulting hepatocyte-like cells displayed specific marker expression and function. These iPS-derived hepatocytes will now be infected with P. cynomolgi sporozoites. This innovative in vitro approach may provide a new and efficient tool for the discovery of antiplasmodial drugs that are active against the quiescent hypnozoites.

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W-2054

TET1-INDUCED HUMAN INDUCED PLURIPOTENT STEM CELLS, TIPS CELLS, AND THEIR IMPROVED DIFFERENTIATION CAPABILITIES FOR NEUROECTODERM AND MESENDODERM

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Human induced pluripotent stem cells (hiPSCs) share key features with mouse epiblast stem cells and are therefore considered to be the in vitro equivalent of primed pluripotent epiblast-stage embryonic cells. However, in practice, hiPSC capacity to differentiate into all somatic cell types varies substantially across different cell lines. Recently, we and others have found that the DNA dioxygenase TET1 bears active role in specifying the epiblast/primed pluripotency. In the current study, we have investigated the effect of TET1 by adding it to Yamanaka's cocktail in hiPSC reprogramming (TiPS cells) and directly compared them to the conventionally-derived hiPSCs (CiPS cells). Additionally, to categorize hiPSCs by their differentiation capabilities or, in other words, quantitatively-tested pluripotency, we have devised a novel evaluation system of differentiation efficiency for neuroectoderm (default-elite) and for mesendoderm (primitive-streak-elite). Interestingly, we observed significant increase in the proportion of cells with high differentiation ability into neuroectoderm among the TiPS cells compared with CiPS ones when we differentiated the cells by default induction to acquire neural properties in a developmentally neutral culture medium. Furthermore, when TiPS cells were differentiated into midbrain dopaminergic progenitor cells, they had equal or higher differentiation capability to those reported using ES cells. These default-elite TiPS cells were screened for primitive-streak-elites by their inductive ability for T/BRA upon mesendoderm-inductive signals which matched the developmental rationale of this lineage. Through these screens, we have found that part of our default-elite TiPS cells also matched the primitive-streak-elite-ness and therefore would fulfill our new definition of "Q-iPSCs" for quantitatively-tested iPSCs for their differentiation capabilities. Analyzing these Q-iPSCs allowed us to unveil part of the underlying mechanism in how TET1 operates to reinforce primed pluripotency in the hiPSC context.

W-2056

IN VIVO GENERATION OF NEURAL STEM CELLS THROUGH TERATOMA FORMATION USING CELLS DEFECTIVE IN IN VITRO DIFFERENTIATION POTENTIAL

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Pluripotent stem cells (PSCs) have the potential to differentiate into all cell types of the body in vitro through embryoid body formation or in vivo through teratoma formation. Recent reports have shown that fully functional and engraftable hematopoietic stem cells could be obtained from induced PSCs (iPSCs) through teratoma formation. These results suggest that an in vivo environment, even non niche-specific, enables pluripotent cells to recapitulate in vivo development into specific cell lineages. Here, we developed an in vivo neural stem cell (NSC) differentiation system through teratoma formation using PSCs. Interestingly, this in vivo differentiation method can be applied to partially pluripotent cells that did not complete the reprogramming process and lack in vitro differentiation potential. In vivo-derived NSCs were similar to brain-derived NSCs in both gene expression profiles and the potential to differentiate into neurons and glial cells. Moreover, they maintained normal karyotype and were not tumorigenic although they were derived from tumor tissue, teratoma. Based on these results, we suggest that teratoma or an in vivo environment, although not niche-specific, endow pluripotent cells with the ability to recapitulate the required NSC niche. In addition, this in vivo differentiation system that mimics 3D in vivo environment will be a powerful method for generation of specific cell types that are difficult to obtain by means of in vitro differentiation protocols.

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W-2058

THE IMPACT OF HYPOXIA AND OXIDATIVE STRESS PROTECTION DURING HUMAN HEMATOPOIETIC DEVELOPMENT

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Oxidative stress is detrimental to the function of Hematopoietic Stem Cells (HSCs). During murine embryonic development, a low oxygen concentration (hypoxia) positively regulates the emergence of HSCs. However, similar studies of the developing human embryo are not possible due to ethical reasons. Using directed in vitro differentiation of Pluripotent Stem Cells (PSCs) towards the hematopoietic lineage we explore the involvement of hypoxia, and protection from oxidative stress, during murine and human hematopoietic development and HSC emergence. Previous results from our lab have shown that the Ly6aGFP and Gata2Venus reporters faithfully track the development of mouse HSCs and hematopoietic progenitors in vivo. For the purpose of studying hematopoietic development in a more tractable model system, we use PSC lines genetically modified to report and track blood formation during in vitro differentiation. Using such PSC lines, we investigated the effects of reduced oxygen levels (6% O2 - hypoxia) compared to standard in vitro culture conditions (~ 21% O2 – normoxia). In both murine and human PSC differentiation cultures we report enhanced expression of key genes involved in hematopoietic development. Hypoxic culture conditions increase the frequency of hematopoietic progenitor cells, and a comparative analysis of populations sorted from hypoxic and normoxic cultures demonstrate a several-fold increase in CFU-C capacity of cells generated in hypoxia, suggesting a positive effect on the function of PSC-derived hematopoietic progenitors. Together, these results support the theory that reduced oxygen levels play an important role on in vitro hematopoietic development in both the human and the murine setting. We are currently investigating hPSC-derived cells for markers of hemogenic endothelium and early HSCs. Measurements for intracellular levels of reactive oxygen species, together with an in-depth analysis of mediators involved in the hypoxic response, will allow us to better elucidate the impact of low oxygen on the process of human hematopoietic development and HSC emergence.

W-2060

QKI-6 INDUCES VASCULAR SMOOTH MUSCLE CELL DIFFERENTIATION THROUGH REGULATION OF THE HISTONE DEACETYLASE 7 MRNA SPLICING

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Cardiovascular disease is a major cause of death, characterized by endothelial cell (EC) dysfunction. In parallel, vascular smooth muscle cells (VSMC) are crucial in embryonic development and blood vessel physiology. Cell therapy based approaches, using ECs with SMCs are promising tools in regenerative medicine. SMCs are a key component of tissue-engineered vessels, but sources of isolation are limited. Using a model of induced pluripotent (iPS) stem cell differentiation to VSMCs, we published that the RNA binding protein Quaking (QKI) isoform 5 (QKI-5) is a key regulator of EC differentiation, neovascularization & angiogenesis by direct binding to the 3' UTR of STAT3 & activating vascular endothelial growth factor receptor. As vascular progenitor cells differentiate to ECs or SMCs they could contribute to vessel development in embryos & vascular disease in adults. It's therefore possible that key regulatory genes of vascular development such as QKI can regulate EC & SMC differentiation from the common vascular progenitor. Interestingly, QKI undergoes alternative splicing resulting in several isoforms. In this study, we have robust evidence that QKI-6 is implicated in iPS-SMC differentiation. Experiments show that QKI-6 is expressed in parallel with VSMC markers during differentiation by culture on collagen IV with DMEM+PDGF-BB. If QKI-6 is overexpressed during differentiation, expression of VSMC markers are significantly increased & mirrored in knockdown. Luciferase assays show QKI-6 drives transcriptional activation of SM22 & pericyte marker NG2, indicating that QKI-6 is directing iPSCs to VSMCs. To elucidate the pathway, we have robust evidence that QKI-6 overexpression highly induced Histone Deacetylase 7 (HDAC7) splicing & markers associated in the HDAC7-SMC mechanism, SRF & Myocardin, with knockdown of QKI-6 suppressing their expression. We have previously shown HDAC7 splicing induced SMC differentiation by modulation of the SRF-myocardin complex, suggesting that HDAC7 splicing is crucial for SMC differentiation & vessel formation. Here, mechanisms of HDAC7 splicing during SMC differentiation are elucidated, mediated by RNA binding protein QKI-6. These findings provide a new insight into mechanisms of SMC differentiation holding therapeutic potential for cardiovascular disease.

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W-2062

TRANSFECTION OF PRONEURAL MESSENGER RNAS INTO HUMAN IPSC DRIVES RAPID DIFFERENTIATION TO MIDBRAIN DOPAMINERGIC NEURONS

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In vitro differentiation of human induced pluripotent stem cells (hiPSC) into neurons for disease modeling, drug screening and therapeutic treatment is frequently an unpredictable process producing a range of neural subtypes. Often the procedures to generate specific neuronal populations via morphogenetic signals are variable, inefficient and time consuming. By superimposing transient expression of mRNAs encoding key transcription factors, hiPSC can be more predictably differentiated into desired neuronal subtypes. The selective generation of A10 dopaminergic neurons of the ventral tegmental area (VTA) from a mixed population of Tyrosine Hydroxylase positive neurons will enable the study of behavioral reward circuits in vitro as they relate to drug abuse and addiction. To achieve this aim we have developed a transfection reagent that efficiently targets hiPSC, and shows little cell toxicity with no need for modified ribonucleosides. This allows repeated delivery of a combination of novel proneural transcription factors that drive neuronal differentiation in five days. Further, we have optimized the timing of external morphogen signals to improve the selectivity for A10 VTA neurons over the more widely studied A9 Substantia Nigra neurons relevant for Parkinson's Disease research. Being able to precisely control differentiation into these two dopaminergic neuronal cell populations should enable the reproducible production of hiPSC models to better study addiction and motor tremor disorders.

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W-2064

EPIGENOMIC AND TRANSCRIPTOMIC ANALYSES IDENTIFY MISSING FACTORS IN HPSC-DERIVED HEPATOCYTES

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Human pluripotent stem cells (hPSCs) have the potential to differentiate into any tissue of the human body, thus offering an unlimited supply of cells for disease modelling, cell therapies and drug screening. The differentiation ability of hPSCs has been previously exploited as a tool to produce hepatocytes for disease modelling and regenerative medicine applications. However, generating hepatocytes that display the functional characteristics of adult primary hepatocytes, including the full spectrum of drug metabolism, remains a major challenge. Here, we performed epigenetic and transcriptomic analyses to identify the "missing factors" in hPSCs-derived hepatocytes to be fully functional. We performed RNA-seq and H3K27ac ChIP-seq comparison between hepatocyte-like cells differentiated from hPSCs for up to 33 days and primary hepatocytes isolated from organ donors. These analyses showed key differences in transcription profiles confirming the immature phenotype of hPSC-derived hepatocytes. Of particular interest, 4631 genes were found to be downregulated in hPSC-derived hepatocytes as compared to primary hepatocytes, which substantially included not only genes involved in drug metabolism, but also a cohort of transcription factors. Concordantly, H3K27ac ChIP-sequencing analyses showed that around 10.000 genomic regions enriched in H3K27ac in primary hepatocytes, lack this histone modification in hPSC-derived hepatocytes, thus suggesting that a proportion of enhancers of genes involved in drug metabolism is epigenetically silenced in this population. Altogether, these analyses reveal that the feotal phenotype of hepatocyte-like cells is caused by the absence of key transcription factors and/or epigenetic modifications. Future work will involve the functional validation of these factors and the identification of signalling pathways controlling their expression. Altogether, these results will enable the development of improved methods of differentiation that will allow the production of fully functional hepatocytes from hPSCs. In addition, these findings could be transferred to other cell types generated from hPSCs and thus provide a universal approach for generating mature cell types from human pluripotent stem cells.

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W-2066

IDENTIFICATION AND CHARACTERIZATION OF LIVER PROGENITOR-LIKE CELLS CAPABLE OF LONG-TERM PROPAGATION FROM HUMAN IPSCS

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Self-renewing bi-potent progenitor cells that are primary contributors to liver regeneration following injury have been identified and isolated from adult human liver and stably maintained in culture by stimulation of LGR5 with Rspondin. Here, we aimed to determine the presence of a similar cell type during the course of differentiating human pluripotent stem cells (hPSC) to hepatocyte-like cells (HLC) in vitro and whether such cells could be maintained in conditions used to support adult liver progenitors. A time course analysis of gene expression during the 25-day differentiation of hPSC to HLC revealed that LGR5 was highly induced and peaked at day 7 suggesting the presence of cells responsive to Rspondin / LGR5 signaling. When day 7 cells were cultured in 90% Matrigel with liver expansion media containing Rspondin, spheroids emerged within one week. The spheroid cultures were maintained over 3 months with weekly passaging, indicating the ability to self-renew. Based on IHC staining, spheroids consisted largely of a single layer of cells expressing ductal / progenitor markers SOX9 and CK19. Expression of bi-potential liver progenitor markers CK19, LGR5, HNF4a, EPCAM and SOX9 was maintained over multiple passages. The spheroid cultures also expressed hepatocyte markers albumin, A1AT and AFP, which were upregulated when cultures were switched to hepatocyte differentiation medium. We further characterized day 7 cells by FACS sorting based on EPCAM followed by gene expression analysis and Matrigel culture to determine spheroid-forming capacity. The majority (>90%) of cells were EPCAM positive and expressed liver stem cell markers LGR5, CK19 and EPCAM. Sorted single cells could form spheroids in culture with the EPCAM high fraction containing the highest spheroid-forming activity. Ongoing work includes characterization of spheroid-forming cells at the single cell level and testing the ability to generate both cholangiocytes and hepatocytes in in vitro and in vivo models of liver repair. hPSC derived cells

with liver stem/progenitor-like properties as described here hold great potential for clinical applications.

W-2068

INVESTIGATING THE ROLE OF TBX FAMILY MEMBERS IN HUMAN LIVER DEVELOPMENT

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The transcription factor Tbx3 has been implicated as a key regulator liver development in the mouse. Tbx3 is responsible for expansion of the liver bud and regulating fate decisions of hepatoblasts, bipotential progenitors that give rise to either hepatocytes or cholangiocytes. However, it is unclear whether TBX3 plays a similar role during human liver development. To study the role of TBX3 in the context of human liver development, we have generated a human embryonic stem cell (hESC) line that uses a doxycycline inducible system to exogenously express TBX3. Using CRISPR/Cas9 technology, we have also generated a TBX3 knockout hESC line (TBX3-/-) in the context of the doxycycline inducible line, providing a useful tool for conducting rescue experiments. Upon differentiation of TBX3-/- ESCs towards a hepatic lineage, both with and without doxycycline, we found no significant defect in hepatocyte differentiation compared to control cell lines. A possible explanation is that TBX3 may not regulate early liver specification in humans. We find that TBX2, a member of the TBX transcription factor family that is highly homologous to TBX3, is also expressed during the in vitro differentiation cultures and could be acting redundantly and compensating for the loss of TBX3. To investigate whether this is the case, we are generating a TBX2/TBX3 double knockout hESC lines and are determining whether the loss of both TBX2 and TBX3 results in a defect in hepatocyte differentiation. These experiments will provide novel insight into the mechanisms regulating hepatoblast fate decisions during human liver development.

W-2070

LAMININ ISOFORMS DIFFERENTIALLY REGULATE ECTODERMAL CELL FATE DURING OCULAR CELL DIFFERENTIATION FROM HUMAN IPS CELLS

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Extracellular matrix is important for stem cell maintenance, expansion and differentiation. Laminin, a major protein of basement membrane, removes the need for feeder cells in human pluripotent stem cell culture. Previously, using short fragment of laminin-511 (LN511E8), we have shown the generation of a self-formed ectodermal autonomous multi-zone (SEAM) of ocular cells from human iPS cells, which consists of four concentric zones of cells. However, how these spatially graded structures are formed and whether other isoforms of laminin affect their formation remain unclear. In this study, to examine the effects of laminin isoforms on differentiation of human iPS cells, ocular cell differentiation of human iPS cells was performed on E8 fragment of laminin isoforms. The results showed that typical SEAM structure was formed on laminin-511, however, on laminin-211 and -332, neural crest cells and surface ectodermal cells were predominantly enriched, respectively, resulting in no typical SEAM formation. Particularly, laminin-332 enabled shortening the period for generation and enrichment of human iPS cells-derived corneal epithelial cells. Moreover, we found that laminin isoforms also differentially regulate not only the differentiation process but also human iPS cell behavior before the differentiation, which affects subsequent ectodermal cell differentiation. This partly explains the mechanisms of SEAM formation from human iPS cells. Together, these results demonstrate how ectodermal cell fate is regulated by laminin isoforms during ocular cell differentiation from human iPS cells.

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W-2072

EFFICIENT 3D CULTURE AND RAPID DIFFERENTIATION OF IPS CELLS USING MICRO-FABRIC VESSELS

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3D suspension culture of human induced pluripotent stem cells (hiPSCs) as spheroids is known to be an effective method for large scale bulk cultivation, expansion and differentiation processes. So far, we have developed an easy, reproducible and high-throughput method of uniformly-sized iPSC spheroid formation with maintaining their high pluripotency, viability and growth rate using the unique micro-fabric vessels "EZSPHERE". In this study, we show the effective and rapid differentiation of iPSCs to neural or cardiac cells using 3D suspension culture. The EZSPHERE has a feature of high density micro-wells (200-1,400 μm in diameter and 100-400 μm in depth) micro-fabric on the culture surface of universal plastic dishes or multi-well plates by CO2 laser and followed with low-cell-attachment coating. Therefore, EZSPHERE is suitable for rapid generation (within 3-6 hours) of uniformly-sized cell aggregates in high density. In order to verify effectivity of this unique culture system for differentiation, iPSC spheroids with four different sizes were generated by varying the seeding cell number (125, 250, 500 and 1,000) per micro-well of the EZSPHERE, which were then followed by testing neural lineage differentiation efficiency. As a result, average diameter of the obtained spheroids was able to be controlled by the seeding cell density. Interestingly, it was found that neural stem cell (NSC) differentiation is spheroid size or seeding cell density dependent. Flow cytometry analysis of the NSC makers (e.g., N-cadherin and CD56) at the early stage of the neural differentiation process revealed differentiation is accelerated in the larger spheroids that obtained as the seeding density around 500 and 1,000 cells / micro-well. Furthermore, it was also confirmed that iPSC spheroids formed in the EZSPHERE could proliferate and finally differentiate into the functional dopaminergic neurons. As an example of other germ lineage cell differentiation, over 85% cardiac troponin T (cTnT)-positive cardiomyocytes could be also produced from the hiPSC spheroids generated on the EZSPHERE. These results suggested the micro-fabric vessels, EZSPHERE, is useful for high-throughput generation of iPSC spheroids for not only basic or clinical research, but also for regenerative medicine in the future.

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W-2074

EXPLORATION OF NRF2 PATHWAY ACTIVATION DURING NEURONAL DIFFERENTIATION OF HUMAN NEURAL STEM CELLS

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Organisms are exposed to reactive oxygen species (ROS) from internal metabolism and environmental toxicants. Overproduction or incorporation of free radicals and imbalanced defence mechanisms against oxidative stress (OS) are linked to cell and organ toxicity. Exposure to ROS is counterbalanced by antioxidant defence systems such as the Nrf2/antioxidant response element (ARE) pathway, a prime regulator of endogenous antioxidant responses. ROS are also important regulators of intracellular signalling pathways involved in early neuronal development. The regulation of neuronal redox homeostasis via the Nrf2/ARE pathway in hPSC-derived neurons is not well understood and has major implications for the use of stem cell-based approaches in basic and translational research. We performed comprehensive time-course microarray studies in hPSC-derived long-term neuroepithelial stem cells (lt-NES) differentiated for up to 12 weeks. Concurrent with neuronal and glial maturation we observed expression changes of components of the Nrf2 pathway. Dose-response analysis revealed an increased resistance of more mature cultures to the OS inducers Rotenone and tert-butyl hydrogen peroxide as determined by AlamarBlue assay. To assess if this phenotype is associated with altered Nrf2 signalling, we performed qRT-PCR analyses of downstream targets after treatment with the Nrf2 inducer tert-butylhydroquinone (tBHQ). Interestingly, we observed that induction of the Nrf2 target genes NQO1 and HMOX1 upon 8h of tBHQ treatment decreases with progressing neuronal maturation. In order to assess the role of glia/neuron interactions in the neuronal susceptibility to OS, we established a shared medium co-culture model of lt-NES neurons and hiPSC-derived astrocytes. Following 48h of Rotenone treatment, a cell-type specific response of the Nrf2 pathway was observed with astrocytes displaying more robust downstream target gene expression changes than neurons. However, the co-cultured astrocytes exerted no protective effect on Rotenone-exposed neurons

in this paradigm. Further elucidation of the cell type-specific and maturation-dependent dynamics of the Nrf2 pathway is expected to provide an important basis for interpreting and counteracting OS-mediated effects in disease modelling and cell therapy, respectively.

W-2076

DRIVING THE MYOGENIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS THROUGH THE FORCED EXPRESSION OF KEY TRANSCRIPTION FACTORS OF THE DEVELOPMENTAL PATTERN

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The in vivo cell therapies treating human skeletal muscle degeneration, as well as the creation of in vitro muscle models for biological and biomedical investigations, require a high number of myogenic progenitor cells, not obtainable from adult donors. This problem can be overcome using human pluripotent stem cells (hPSCs), because they ensure large amounts of cells exploitable as transplantable material, possibly patient-specific. However, the existing in vitro myogenic differentiation strategies show low yields and the use of viral vectors makes such cells unsuitable for therapy purpose. In this scenario we aimed at developing an innovative strategy to induce myogenic differentiation of hPSCs using a non-integrative technology. We performed multiple transfections with modified mRNAs (mmRNAs) coding for the key myogenic transcription factors PAX3, PAX7 and MYOD. To this aim we exploited microfluidic platforms, which allow the downscaling of the process in order to perform cost-effective, multiparametric and highthroughput experimental investigations. We demonstrated that MYOD mmRNA delivery to hPSCs is not effective in inducing myogenic differentiation. Indeed, it is compulsory to prime hPSCs towards mesoderm before mmRNAs delivery. We successfully induced early mesoderm modulating Wnt and bone morphogenetic (BMP) pathways signaling, with CHIR33021 and LDN-193189, prior to the transfections. At day 2, we obtained 100% of cells positive to the early mesoderm gene BRACHYURY T (BRYT). Subsequently, seven consecutive transfections were performed on mesoderm-induced cells with PAX3, PAX7 and MYOD. We obtained an increase in endogenous expression of MYF5 1 day after the last PAX3 transfection. Endogenous expression of desmin was observed 4 days after the last transfection with PAX7 and the same result was obtained with MYOD. MYOD mmRNA also stimulated the endogenous expression of myogenin and myosin heavy chain. Ongoing experiments are verifying the efficacy of mmRNA transfections in obtaining a more mature muscle phenotype, also combining together the mmRNAs with a precise timing. Our differentiation strategy proves to be an effective method for the obtainment of myogenic cells for development studies, drug testing, tissue engineering and personalized cell therapy with clinical grade cells.

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PLURIPOTENT STEM CELL: DISEASE MODELING

W-2080

COMBINING GENE EDITING AND HUMAN STEM CELLS TO MODEL DIABETES DUE TO HNF1A DEFICIENCY

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Maturity Onset Diabetes of the Young type 3 (MODY3) accounts for 60% of cases of MODY and is caused by mutations of the HNF1α gene, leading to impaired β-cell mass and reduced insulin production. HNF1α is a transcription factor playing an important role in β-cell development, proliferation, viability and insulin release. It is likely that subtler derangements of the function of these genes and their pathways account for some proportion of susceptibility to more prevalent forms of diabetes. By generating insulin-producing β-cells from MODY3-patient's iPSCs and HNF1a mutant hESCs, we characterized the cellular and molecular defects caused by HNF1a deficiency to better understanding of the mechanisms underlying β-cell development, function and survival. To this purpose, by using genome-editing CRISPR/Cas9 technology, we have generated mutation-corrected and homozygous mutant patient cell lines for HNF1a. Gene manipulation of HNF1a by CRISPR/Cas9 technology was also performed in a GFP-reporter hESCs cell line that harbors a GFP gene knock-in in one INS locus to enable isolation of viable INS-GFP+ cells in vitro and in vivo. We generated glucose-responsive pancreatic β-cells from these isogenic genotypes and characterized the cellular and molecular phenotypes caused by HNF1a deficiency. Our data show that HNF1a mutant β-cells display increased insulin content and reduced basal insulin secretion and that correction of MODY3 patient cell lines can reverse this phenotype in vitro. Mutant β-cells also have impaired insulin secretion in vivo and fail to restore normoglycemia in a STZ-induced diabetes mouse model. However, injection of anti-diabetic drug glibenclamide can partially improve the secretion defects of the HNF1a mutated β-cells. Finally, global and single-cell RNAseq from INS-GFP+ sorted cells indicate that genes involved in β-cell

secretion and insulin packaging are major players in this phenotype. These studies demonstrate that stem cell derived β-cells grafted into mice are reliable and faithful model and provides a proof of principle for gene therapy combined with cell therapy for diabetes.

W-2082

CELL-TYPE DEPENDENT ALZHEIMER'S DISEASE PHENOTYPES: PROBING THE BIOLOGY OF SELECTIVE NEURONAL VULNERABILITY

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Alzheimer's disease (AD) is a highly prevalent neurodegenerative disorder characterized by extracellular plaques composed of amyloid β-protein (Aβ) and intraneuronal tangles consisting of altered forms of the Tau protein. AD induces progressive memory impairment and cognitive decline in the virtual absence of motor and sensory deficits during its early and middle course. A major unresolved question is the basis for this selective neuronal vulnerability: why are cells in distinct brain regions differentially susceptible to neurodegeneration in AD? Aβ, which plays a central role in AD pathogenesis, is generated throughout the brain, yet some regions outside of the limbic and cerebral cortices such as midbrain, hindbrain and spinal cord are relatively spared from Aβ plaque deposition and synapse loss. Here, we examine neurons derived from induced pluripotent stem cells (iPSCs) of patients harboring an AD-causing APP mutation (APPV717I) to quantify AD-relevant phenotypes following directed differentiation to rostral fates (vulnerable) and caudal fates (relatively spared) in AD. We quantitatively compare control and APPV717I iPSCs and find that caudal neurons differ from rostral neurons in both their generation of and responsiveness to Aβ species, with rostral neurons being more sensitive to fAD mutation than caudal neurons. APPV717I neurons directed to caudal neuronal fates generated Aβ with a lower 42:40 ratio and higher 38:42 ratio than rostral neurons. Further, we show that APPV717I neurons express higher levels of total and phospho-Tau proteins relative to control neurons when directed to a rostral neuronal fate, but not when directed to a caudal neuronal fate. Finally, we demonstrate that neurons of these different cell fates respond differentially to soluble extracts of clinically and neuropathologically typical 'sporadic' AD brains. The AD extracts induce an elevation in the phosphorylation of Tau in forebrain neurons, and this is specifically dependent upon the Aβ present in these extracts. However, when exposed to the same AD extracts, Tau phosphorylation is not affected in neurons directed to caudal fates. Taken together, our results suggest that both APP processing and Tau homeostasis are differentially altered between neuronal subtypes that are relatively vulnerable or resistant to AD.

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W-2084

GENERATION OF GENE-CORRECTED IPSC FROM PATIENT-DERIVED IPSC WITH FAMILIAL PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disorder caused by loss of nigrostriatal dopaminergic neurons. Leucine-rich repeat kinase 2 (LRRK2) is the causative molecule of the autosomal dominant hereditary form of PD, PARK8, which was originally defined in a study of a Japanese family (the Sagamihara family) harboring the I2020T mutation in the kinase domain. Although a number of reported studies have focused on cell death mediated by mutant LRRK2, details of the pathogenetic effect of LRRK2 remain completely unknown. Recently, we have reported induced pluripotent stem cells (iPSC) derived from patient with I2020T LRRK2 in the Sagamihara family (I2020T LRRK2-iPSC) replicate to some extent the pathologic phenotype evident in the brain of PD patients. In this study, to elucidate the mechanism of neurodegeneration in PD caused by LRRK2 mutation, we generated gene correction of iPSC from I2020T LRRK2-iPSC using TALEN (transcription-activator-like effector nuclease)-mediated genome editing. To examine

gene-corrected iPSC (TALEN-iPSC) lines established, we performed genomic DNA sequencing of exon 41 of the LRRK2 gene. The TALEN-iPSC lines showed the same normal sequence of LRRK2 gene but not I2020T mutation. Next, to evaluate the differentiation efficiency of TALEN-iPSC lines established and I2020T LRRK2-iPSC and control iPSC-derived neurospheres were allowed to differentiate to neurons. After 15 days, the differentiated cells were examined for βIII-tubulin (a neuron-specific marker) and tyrosine hydroxylase (TH) (a dopaminergic neuronal marker). We found that >65% of the differentiated cells were positive for βIII-tubulin, and that approx. 15% of neurons from all the iPSC clones were TH-positive. Finally, to investigate I2020T LRRK2 affect neurite outgrowth, we analyzed by immunostaining. After 15 days in vitro, neurite outgrowth of I2020T LRRK2 iPSC-derived neurons were shorter than those of control iPSC-derived neurons. On the other hand, TALEN-iPSC-derived neurons rescued this phenotype to a level equal to the control iPSC-derived neurons. These results indicate that gene-corrected iPSC may serve as a useful model to elucidate PD pathogenesis.

W-2086

CRISPR KNOCK-OUT OF ALPHA-SYNUCLEIN IN PATIENT-DERIVED PLURIPOTENT STEM CELL MODEL OF PARKINSON'S DISEASE

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The accumulation and aggregation a-synuclein protein (a-syn) is a critical event in Parkinson's disease (PD) pathophysiology, impairing neuronal function and contributing to dopaminergic neuronal cell death. The pathogenic genomic triplication of the alpha-synuclein (SNCA) gene (chromosomal locus 4q21, size 1.7Mb) in patients results in early onset rapidly progressive parkinsonism with diffuse Lewy body pathology and severe autonomic involvement, suggesting a direct link between increased gene expression of wild-type a-syn and disease development. We have previously shown that overexpression of a-syn as it related to the SNCA genomic triplication is linked to increased susceptibility for oxidative stress and impairment of neuronal maturation in patient-derived fibroblasts or induced pluripotent stem cell (iPSC) models. The goal of this study was to combine iPSC technology with gene editing to establish isogenic cellular tools which express varying SNCA gene copy numbers. With this set of new cell lines, we are able to address what are the physiological and detrimental effects of varying a-syn levels, thus greatly simplifying the experimental paradigm that arises when overexpressing proteins or downregulating gene expression. We have generated CRISPR tools to introduce double-strand breaks in the first coding exon of SNCA gene. Human iPSCs from a SNCA triplication carrier were growth adapted to single cell cloning and were transfected with the CRISPR constructs several rounds before genotyping of individual clones. We generated 11 clones with different mutant alleles relating to 4 knockout (KO), 3KO, 2KO, 1 KO. The resulting iPSCs were karyotypically normal and expressed pluripotency markers. mRNA expression decreased corresponding to the number of functional copies of the SNCA gene. Dopaminergic neurons derived from these isogenic lines are analyzed for viability, differentiation potential and morphological as well as physiological changes to evaluate the effect of different 'gene doses' of alpha-synuclein. Here, we present a unique in vitro model system to study the impact of a-syn in an isogenic background. This system will be extremely useful for the study of a-syn associated pathways, drug screening, and the pharmacological modulation of a-syn levels in PD pathophysiology.

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W-2088

CO-EXPRESSION AND PREDICTIVE NETWORK BASED KEY DRIVER ANALYSIS OF INSULIN RESISTANCE IN HUMAN IPSC LINES

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Insulin resistance (IR) precedes the development of type II diabetes and is a serious risk factor for the development of cardiovascular disease. While GWAS studies have helped to uncover new loci associated with the development of type II diabetes, most of these signals are associated with pancreatic function and insulin secretion, giving little insight about the mechanism that leads to the development of IR. Thus, new approaches are necessary to explore the genetic architecture and signaling pathways that lead to IR. To that end, we have generated a large-scale iPSC library from individuals reflecting the broad spectrum of insulin response in human populations, measured by steady-state plasma glucose (SSPG) levels. RNAseq analysis of 328 iPSC clones (103 individuals) has identified differentially expressed genes and pathways associated with energy metabolism and insulin signaling between IR and insulin sensitive (IS) iPSC lines. Analysis of the co-expression architecture has described several

sub-networks (modules) of interest in both IR and IS networks that are associated with cholesterol and lipid metabolism, mitochondrial function and glycolysis. These processes are believed to have an intimate relationship with IR development. Using predictive network modeling we have identified a set of key driver genes that potentially control and regulate these co-expression modules. Interestingly, one of our top hits is 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR), which is a known target for statins. Moreover, recent studies have raised concerns about the possibility that statin treatment increases type II diabetes risk. Functional validation in human adipocytes and skeletal muscle cells will help to elucidate the relevance of our key driver candidate genes in the context of the initial steps that lead to the development of IR.

W-2090

CRISPR-MEDIATED MODELING AND FUNCTIONAL GENOMIC INVESTIGATION OF RECIPROCAL MICRODELETION/ MICRODUPLICATION SYNDROMES

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Recurrent microdeletion and microduplication syndromes (rMDS) are among the most common causes of human neurodevelopmental and psychiatric disorders. These recurrent rearrangements are mediated by non-allelic homologous recombination (NAHR), which occurs between two highly homologous flanking segmental duplication (SD) sequences and can result in either copy loss (microdeletion) or the reciprocal copy gain (microduplication) of an identical intervening genomic segment. Unfortunately, each individual recurrent genomic disorder is relatively rare, and accurate modeling of their impacts in model systems represents a major challenge. The capacity to mimic these rMDSs in an otherwise isogenic pluripotent stem cell can provide an invaluable tool for modeling rMDS in humans. As a proof-of-principle, we describe a CRISPR/Cas9 genome engineering method, Single-guide CRISPR/Cas targeting Of Repetitive Elements (SCORE), which targets homologous sites in each flanking SD to efficiently model NAHR-mediated rMDS. Our methods successfully generated reciprocal copy number variation (CNV) of 16p11.2 and 15q13.3 rMDS regions, 740 kb and 1,989 Mb respectively, which are common rMDSs that have been associated with developmental delay, autism spectrum disorders and schizophrenia. The method that mediates NAHR in vivo is reproducible, and RNA sequencing reliably clusters transcriptional signatures from human subjects with in vivo CNVs and their corresponding in vitro models. To further explore the transcriptional networks and pathways implicated in 16p11.2 rMDS (OMIM611913), which features autism and several other phenotypes, we then perform comprehensive transcriptome analysis of tissues from 16p11.2 mouse models and multiple cell lineages derived from the patient and CRISPR iPSC lines harboring 16p11.2 CNV. Our data suggest that this SCORE approach has broad applicability for genomic disorders and, with in-depth functional genomic analysis and neuron functional measurements may allow us to uncover the driver genes and networks in 16p11.2 rMDS (OMIM 611913) and 15q13.3 rMDS (OMIM 612001) as clues to the molecular pathogenesis of autism spectrum disorders and schizophrenia.

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W-2092

DETERMINING GENETIC CONTRIBUTORS TO ZAAT-DRIVEN PROTEOTOXICITY USING CRISPR-EDITED SYNGENEIC PATIENT-IPSCS

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Alpha-1 antitrypsin deficiency (AATD) is a monogenic disorder associated with both lung and liver disease. The most common disease variant, the Z-mutation, results from a single base pair mutation that in turn causes protein misfolding and polymerization within the ER of hepatocytes. In 10% of patients the protein accumulation results in cirrhosis; however, the link between misfolding and development of end-organ damage remains poorly understood. Human induced pluripotent stem cells (hiPSCs) have been shown to recapitulate critical aspects of AATD liver disease pathogenesis and can model patient specific phenotypes. We hypothesize that hiPSCs derived from AATD patients will provide insight into pathways associated with liver disease susceptibility and allow study of genetic disease modifiers. To test this hypothesis we have utilized the CRISPR endonuclease system to correct the Z-mutation to generate syngeneic hiPSC clones allowing us to

highlight changes resulting directly from the mutant protein. gRNA sequences were administered together with a ssODN repair template to accomplish scarless genome editing without antibiotic selection. Using this approach we have achieved bi-allelic correction in diseased "PiZZ" hiPSCs with an overall HDR efficiency of 19.8%. Syngeneic hiPSCs were then differentiated to the hepatic stage and analyzed for changes in intracellular AAT accumulation and gene expression. The CRISPR corrected "PiMM" hiPSC-hepatic cells demonstrated significant reduction in intracellular AAT accumulation with a concomitant increase in protein secretion relative to their parental "PiZZ" lines. Additionally markers of ER stress were significantly reduced including the ER-stress specific caspase 4. Microarray-based analysis of the global transcriptome comparing diseased PiZZ to corrected PiMM hiPSC-hepatic cells from two genetically distinct hiPSC pairs demonstrated 60 differentially expressed genes commonly shared (adjusted FDR-adjusted $p < 0.1$). These findings demonstrate that following CRISPR correction the resultant syngeneic hiPSCs lose the pathognomonic AAT protein retention, which is associated with significant changes in gene expression. Further analysis of these changes could provide key insight into contributors to AATD-liver disease susceptibility.

W-2094

MODELLING LUNG DISEASE IN ALPHA-1 ANTITRYPSIN DEFICIENCY USING HUMAN ALVEOLAR EPITHELIAL CELLS DERIVED FROM GENE-EDITED INDUCED PLURIPOTENT STEM CELLS

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Type II alveolar epithelial cells (AEC2) play key roles in the response of lung epithelial cells to injury and are involved in the pathogenesis of disease such as COPD and IPF. These difficult to isolate cells are capable of producing alpha-1 antitrypsin (AAT) but little is known about their role in disease pathogenesis in alpha-1 antitrypsin deficiency (AATD). A single base pair mutation of the SERPINA1 gene leads to low levels of circulating AAT protein causing emphysema in AATD. We hypothesise that AEC2s play an intrinsic role in AATD pathogenesis and therefore we sought to engineer a novel in vitro model to enable the derivation of lung epithelial cells from patients with AATD. We targeted fluorescent reporters into the endogenous NXK2-1 and surfactant protein C (SFTPC) loci of wild type human iPSC. Using a directed differentiation approach that recapitulates embryonic development we generated lung progenitors followed by putative AEC2s and characterization of these cells was carried out by RNA-seq, intracellular flow cytometry and ELISA for AAT, and electron microscopy for cellular ultrastructure. Sorted lung progenitors (NKX2-1+/SFTPC-) as well as more differentiated distal alveolar epithelial cells (SFTPC+) iPSCs express AEC2-specific genes by RNA-seq, together with increased expression of AAT mRNA and protein, compared to undifferentiated cells. ELISA shows significant levels of AAT secretion from cells at this developmental stage. In addition, iPSC-derived SFTPC+ contain cytoplasmic inclusions with the ultrastructural characteristics of lamellar bodies and Western blotting confirms functional processing of SFTPB protein into the 8kD mature form. Finally, we targeted a fluorescent reporter into the SFTPC locus of an iPSC line generated from an individual homozygous for the SERPINA1 Z gene mutation (PiZZ) before CRISPR-based gene editing to correct the PiZZ genotype to syngeneic PiMM iPSC. In summary, we find that differentiation of PiMM iPSC lines can generate AEC2-like cells which contain functional lamellar bodies similar to those found in mature AEC2s, and that these cells express AAT at significant levels at both mRNA and protein levels. We have also created gene corrected syngeneic clones from individuals with AATD for future studies aimed at determining the role of AEC2s in AATD lung disease.

W-2096

DEVELOPING A NOVEL STEM CELL BASED SYSTEM TO STUDY GDNF INDUCTION IN THE RETINA

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Retinal degenerative disorders, such as retinitis pigmentosa and age-related macular degeneration, are characterized by the progressive and irreversible loss of photoreceptors. Previous studies have shown that growth factors (GF) can postpone and rescue retinal neurons from death. The clinical trials of GF, delivered as recombinant proteins, have not yet resulted in the effective therapy. Endogenous neuroprotection strategy is an alternative: with this approach GF are induced by small molecules in the host tissue. Here we describe a novel in vitro system to search for the molecules that can upregulate GDNF in the retina of the eye. The retinal tissue is differentiated from mouse pluripotent stem cells using three-dimensional. With this approach we can derive structures, representative of all retinal cell types. ELISA was used to quantify the Gdnf and Pedf production at different stages of development and the dose-dependent induction of GDNF by Amitriptyline and Dopamine. The time-course studies showed gradual increase in the production of GDNF and PEDF: from 1 to

8 pg/ml and from 80 to 166 pg/ml, at day 0 and day 35, respectively. The GDNF induction studies showed 2.1 fold induction by 2.4uM Amitriptyline and 2.7 fold induction by 2.4uM dopamine hydrochloride. The EC50 were calculated as 0.75 uM and 0.5 uM for Amitriptyline and Dopamine hydrochloride, respectively. To increase the throughput for drug discovery we have generated a reporter cell line, where Luciferase and GFP are expressed under the control of the Gdnf promoter. Using CRISPR/Cas9 technology the last exon of Gdnf was cut out and replaced by a cassete carrying the last exon of Gdnf without a stop codon, followed by Renilla Luciferase and GFP genes. All three genes were separated by T2A self-cleaving peptide sequences, which ensures the synthesis of the wild-type, untagged Gdnf protein. Renilla Luciferase was chosen as a reporter to quantify the levels of expression of Gdnf after drug treatment and GFP was used to facilitate selection of cells where the cassette integration had occurred. Mouse retinal neuroblasts were used to create the reporter cell line and initial experiments show that 3% of cells express GFP 2 weeks after the transfection. The tools described above would help us in the identification of compounds, capable of GDNF induction in the retina.

W-2098

MODELING BRCA1-INDUCED BREAST CANCER WITH HUMAN IPS CELLS

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Germline mutations in the BRCA1 gene predispose to early onset breast cancers, with a lifetime risk of 80%. Mutations in BRCA1 have diverse effects on the BRCA1 protein, which is involved in various cellular processes including DNA damage repair pathways. In addition to known pathogenic mutants, the BRCA1 gene has many Variants of Unknown Significance (VUSs), leading to difficulties in clinical interpretation and prognosis. It is still unclear whether BRCA1 acts as a tumour suppressor, or whether mutations are dominant-negative, causing functional disruptions which can be observed in heterozygous cells. There are inconsistencies between published studies on the impact of BRCA1 variants on DNA damage response. Here, we aim to evaluate induced Pluripotent Stem (iPS) cells as a model to study the functional consequences of known pathogenic mutants and VUSs. Tissue samples have been collected from patients undergoing risk-reducing mastectomy or reduction mammoplasty. iPS cells were derived and characterized from fibroblasts of known BRCA1 pathogenic mutation (e.g. C61G) or VUS (described clinically as likely non-pathogenic) carriers, and non-mutant BRCA1 healthy controls. These lines have been characterized for pluripotency (including teratoma formation), and for any genomic instability due to reprogramming, by whole-exome sequencing. To evaluate the pathogenic effect of BRCA1 mutations, different DNA damage repair assays have been carried out. Both known pathogenic and VUS lines presented a significantly higher number of chromosomal aberrations after gamma-irradiation compared to healthy controls, together with deficiencies in the Homologous Recombination (HR) repair pathway. Also, the level of damage, as assessed by measuring gγH2AX foci formation, was significantly lower in known pathogenic lines than control lines. Since BRCA1 mutations specifically cause breast cancers, assessment of mammary epithelial differentiation and tumorigenic potential of heterozygous mutants is being carried out. Taken together, our data suggest that DNA damage repair is impaired in iPS cells carrying BRCA1 heterozygous mutations. Overall, iPS cells will be of major interest for the study of VUS pathogenicity, and potentially, for the testing of novel therapies.

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W-2100

FUNCTIONAL HUMAN IPS-ECS FROM DIABETIC PATIENTS PROVIDE BASIS FOR IN VITRO MODEL OF DIABETIC RETINOPATHY

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Diabetes affects over 400 million people worldwide. Diabetic patients develop macro and/or microvascular complications that highly increase the morbidity and mortality of the disease. Endothelial cell (EC) dysfunction is known to be the triggering event of complications like Diabetic Macular Edema (DME), although the mechanisms that underlie the process remain to be understood. Intriguingly, DME patients have different responses to the anti-VEGF gold standard treatment, and only ~50% benefit from it. Induced pluripotent stem cells (iPSCs) are powerful tools for disease modelling, drug screening and personalised medicine. However, there is a need for optimisation of the processes of iPSCs generation to make them more efficient, patient compliant, cost effective and faster. In our group,

we have generated and characterised iPSCs from peripheral blood mononuclear cells (PBMCs) obtained from healthy donors and diabetic patients. In order to establish an in vitro model that mimics the endothelium of these patients, we have subjected the patient specific iPSCs to EC differentiation. For that we have temporarily activated the Wnt signalling pathway and subsequently directed the cells from early mesoderm to EC lineage. On day 6, around 50% of the cells were CD144 positive and were selected using Magnetic Activated Cell Sorting and characterised as endothelial-like cells: the cells express EC-specific markers such as CD144, CD31 and ZO1, they uptake Ac-LDL and they form tubes in vitro and in vivo. These iPS-ECs have provided the basis for in vitro assessment of patient-specific microvascular EC function, including the study of the activation of key pathways (protein assays and phospho-proteomic arrays), the assessment of permeability properties and the identification of genes expressed differently between healthy and diabetic patient groups (large screening profile). In summary, we have efficiently generated a robust in vitro patient specific model of the diabetic endothelium from PBMCs. We have also been able to compare patient-specific iPS-ECs under different stimuli and find key differences that might relate to their different responsiveness to anti VEGF treatment. Ultimately, this model can be used to study other micro/macrovascular complications that arise from diabetes and other endotheliopathies.

W-2102

A STEM CELL REPOSITORY FOR HUMAN NEUROLOGICAL AND PSYCHIATRIC DISEASE RESEARCH

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Established in 1998, RUCDR Infinite Biologics (RUCDR, www.rucdr.org) is the world's largest university-based integrated cell and DNA repository, assisting researchers throughout the world by providing the highest quality biomaterials, technical consultation, and logistical support. Its services include sample collection and bioprocessing (i.e., blood fractionation, nucleic-acid extraction, cell-line creation, etc.) and analytical services such as gene expression, sequencing, and genotyping. RUCDR offers comprehensive stem cell culture services that include the reprogramming of source cells such as skin fibroblasts and blood cells to yield induced pluripotent cells (iPSC) and genome editing using CRISPR/CAS technology. In addition, RUCDR performs a complete range of assays to characterize iPSCs to assess their quality, pluripotency, germline potential and genomic stability, and distributes a cGMP grade iPSC line. RUCDR has been awarded cooperative grants from the NINDS and NIMH to establish stem cell repositories that provide high quality patient and control iPSCs and somatic cells from a wide range of disorders. The NINDS Human Cell and Data Repository (NHCDR) has 169 fibroblast and 72 induced pluripotent stem cell (iPSC) lines for Alzheimer's Disease (AD), Amyotrophic Lateral Sclerosis (ALS), Ataxia-telangiectasia, Frontotemporal Lobar Degeneration (FTD), Huntington's Disease (HD), Parkinson's Disease (PD), and healthy controls. These human cell lines are available to both academic and industry investigators for research purposes and can be ordered through the online NHCDR catalog (https://stemcells.nindsgenetics.org/). The NIMH Stem Cell Center has 684 fibroblast and 292 induced pluripotent stem cell (iPSC) lines in support of investigators engaged in stem cell-based research relevant to mental disorders, including but not limited to anxiety disorders, attention deficit hyperactivity disorder, autism spectrum disorders, bipolar disorder, borderline personality disorder, depression, eating disorders, obsessive-compulsive disorder, panic disorder, post-traumatic stress disorder, and schizophrenia. The NIMH cell line collection administered through the Stem Cell Center can be accessed through the menus at https://www.nimhgenetics.org/stem_cells/.

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W-2104

NEURONS DERIVED FROM PATIENTS WITH BIPOLAR DISORDER DIVIDE INTO INTRINSICALLY DIFFERENT SUB-POPULATIONS OF NEURONS, PREDICTING THE PATIENTS' RESPONSIVENESS TO LITHIUM

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Bipolar disorder (BD) is a progressive psychiatric disorder with more than 3% prevalence worldwide. Affected individuals experience recurrent episodes of depression and mania, disrupting normal life and increasing the risk of suicide greatly. The complexity and genetic heterogeneity of psychiatric disorders have challenged the development of animal and cellular models. We recently reported that hippocampal

dentate gyrus (DG) neurons differentiated from induced pluripotent stem cell (iPSC)-derived fibroblasts of BD patients are electrophysiologically hyperexcitable. Here we used iPSCs derived from Epstein-Barr virus (EBV)-immortalized B-lymphocytes to verify that the hyperexcitability of DG-like neurons is reproduced in this different cohort of patients and cells. Lymphocytes are readily available for research with a large number of banked lines with associated patient clinical description. We used whole cell patch clamp recordings of over 460 neurons to characterize neurons derived from control individuals and BD patients. Extensive functional analysis showed that intrinsic cell parameters are very different between two groups of BD neurons, those derived from lithium (Li)-responsive (LR) patients and those derived from Li non-responsive (NR) patients, which led us to partition our BD neurons into two sub-populations of cells and suggested two different sub-disorders. Training a Naïve Bayes classifier with the electrophysiological features of patients whose responses to Li are known allows for accurate classification with more than 92% success rate for a new patient whose response to Li is unknown. Despite their very different functional profiles, both populations of neurons share a large, fast after-hyperpolarization (AHP). We therefore suggest that the large, fast AHP is a key feature of BD and a main contributor to the fast, sustained spiking abilities of BD neurons. Confirming our previous report with fibroblast-derived DG neurons, chronic Li treatment reduced the hyperexcitability in the lymphoblast-derived LR group but not in the NR group, strengthening the validity and utility of this new human cellular model of BD.

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W-2106

NOVEL CIRCADIAN RHRTHM SLEEP DISORDER MODEL USING PLURIPOTENT STEM CELLS

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The word“Circadian” comes from the Latin circa (about) and dies (day). Many physiological functions have diurnal variation that follows an approximately 24-hour cycle and is regulated by an endogenous circadian clock; dysregulation of these functions leads to circadian rhythm sleep disorders. Recently, individual differences in circadian period length have been shown to be associated with circadian rhythm sleep disorders. For example, familial advanced sleep phase syndrome (FASPS) is an autosomal dominant disease characterized by a natural tendency to go to sleep and wake up at times considered earlier than normal. This phenotype is caused by period shortening of the circadian rhythm due to a point mutation of the circadian clock gene Per2. However, no previous experimental systems have succeeded to recapitulate the period shortening seen in FASPS in vitro. In this study, we modeled circadian rhythm sleep disorders in vitro and established the versatile experimental method. We focused on the fact that circadian oscillation of ES cells appears with differentiation. With broad parameter search, we established ES differentiation system to detect precise period length and succeeded in detecting 10 peaks of oscillation in real-time bioluminescence monitoring. When we introduced wild type and FASPS (Familial Advanced Sleep Phase Syndrome) mutant of Per2 into Rosa26 locus of Per2(-/-) ES cells with targeted genome editing and differentiate them, we could successfully detect period shortening in a FASPS mutant, for the first time at the cellular level. As our system was able to produce the phenotypes of other Per2 mutations, our system proved to be versatile. Moreover, we could elucidate the mechanism of Per1-Per2 cooperation using this new system. In conclusion, we established a novel circadian rhythm sleep disorder model using pluripotent stem cells, first in vitro that recapitulated the period shortening seen in FASPS. The study suggests that this system would be applicable to real-time bioluminescence analysis using patient-derived iPS cells and may also reveal the predisposition of circadian disorders.

W-2108

THE ROLE OF THE HISTONE DEMETHYLASE KDM5C IN THE DEVELOPMENT OF INTELLECTUAL DISABILITY

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The field of epigenetics is reshaping the understanding of neurological disorders. Recent investigations of the brain epigenome emphasise the importance of chromatin regulators in brain function and development. An important example is the chromatin eraser gene KDM5C. Mutations in KDM5C, a histone H3K4me3/2-specific demethylase, are associated with X-linked intellectual disability (XLID), a prevalent brain disorder, and other neurological disorders such as Autism and Huntington’s disease. The causality and the mechanistic insights however still remain elusive. Our research presented here aims to reveal how KDM5C mutations contribute to the development of XLID. To achieve this

goal induced pluripotent stem cells (iPSCs) generated from XLID patient fibroblasts and isogenic control cells were differentiated into cortical neurons (2D cultures) and cerebral organoids (3D cultures). Careful study of morphology, function, genome-wide expression profiling as well as the analysis of the chromatin state during neuronal differentiation revealed that cells and cerebral organoids containing mutations in KDM5C do not follow a normal neuronal developmental program. We observed an altered expression timing and distribution of essential neuronal genes such as Pax6, Tbr2, Tuji, Ctip2 and Satb2 accompanied by cellular organisation differences in 2D and cerebral organoid (3D) cultures. Here we therefore present novel insights not only into disease etiology but also provide important understanding of how KDM5C, a chromatin eraser gene contributes to brain development.

W-2110

ESTABLISHING A MOLECULAR PHENOTYPE FOR ANGELMAN SYNDROME INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS

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Angelman Syndrome (AS) is a neurodevelopmental disorder occurring approximately once in every 15,000 live births for which there is currently no cure. AS is characterized by severe seizures, absent speech, motor dysfunction and ataxia, profound intellectual disability, and happy demeanor. Loss of expression of the maternal copy of UBE3A, a gene regulated by tissue-specific genomic imprinting, causes AS. UBE3A encodes an E3 ubiquitin ligase that may also act as a transcriptional co-activator. We have derived induced pluripotent stem cells (iPSCs) from an AS patient with a missense mutation in UBE3A (F583S). The mutation is located in the HECT domain of the protein, which is the domain that confers ubiquitin ligase activity. This mutation does not affect UBE3A RNA or protein levels, but causes a reduction in the protein's ubiquitin ligase activity, as demonstrated by in vitro ubiquitination assays. Using CRISPR/Cas9-mediated genome editing, we have corrected this point mutation in the AS F583S iPSCs, generating an isogenic control iPSC line (F583S-CTRL). Both F583S and F583S-CTRL iPSCs have been successfully differentiated into 12-week forebrain neurons. We present here preliminary electrophysiological data from these two neuron lines. Whole-cell patch-clamp recordings were performed on 12-week F583S and F583S-CTRL neurons to determine the extent to which these AS iPSC-derived neurons recapitulate the electrophysiological phenotypes seen in other AS iPSC-derived neuron lines. Strand-specific mRNAseq was also performed on each neuron line to establish a molecular transcriptome phenotype for AS iPSC-derived neurons. This transcriptome data provides us with a quantifiable phenotype that we can use to assess changes in AS neurons following pharmacological treatment, for example, or to identify transcripts that are changed not only in this specific AS neuron line but in other AS iPSC-derived neurons.

W-2112

CRISPR/CAS9 PLATFORM FACILITATES EFFICIENT AND PRECISE DISEASE MODELING IN HUMAN INDUCED PLURIPOTENT CELL LINES

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The advent of hiPSC (Human Induced Pluripotent Stem Cells) and CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/ Associated Nuclease Protein-9) technologies has been heralded as a genetic revolution for gene therapy and regenerative medicine. Researchers are exploring the genetics and mechanisms of diseases by introducing mutations or correcting existing mutations in patient-derived iPSCs by using CRISPR/Cas9 techniques for a new era in personalized medicine. While CRISPR/Cas9 techniques has been widely and rapidly adapted for gene manipulation in various organisms in just a few years since its inception, the genome editing in hiPSCs has been slower to develop given the sensitive nature of these cells and the unique skills required to handle them. Furthermore, accurate disease modeling requires more dependency on homology directed repair (HDR) of the CRISPR/Cas9 nuclease system as well as precise single or bi-allelic manipulations. Various parameters affect the outcome of CRISPR-editing in iPSCs such as gRNA design, transfection of CRISPR elements, cell clone selection, etc. Here, we present some case studies to demonstrate how optimization of both iPSC and CRISPR/Cas9 protocol allows for efficient mutation models, including defined heterozygous and homozygous mutations, large fragment knock-in and conditional/ inducible expression models.

REPROGRAMMING

W-2116

DIRECTLY REPROGRAMMED HUMAN NEURAL PRECURSOR CELLS: A NOVEL POPULATION OF CELLS THAT PROMOTE REPAIR AND FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD INJURY

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Human neural precursor cells (hNPCs) represent an exciting therapeutic strategy to repair the traumatically injured spinal cord, however, existing sources of hNPCs (iPSC- and ESC-derived) have translational limitations. Here we describe a novel and highly-efficient technique to directly reprogram somatic cells into hNPCs (drNPCs) without gene insertion or intermediate cell steps. The drNPCs have been extensively characterized in vitro and found to express markers of NPCs but not markers of pluripotency. The cells were further optimized for spinal cord injury (SCI) by enrichment to oligodendrogenic drNPCs (O-drNPCs) which demonstrate a differentiation bias towards an oligodroglial lineage in vitro and in vivo. drNPCs and O-drNPCs were transplanted into a clinically-relevant contusion-compression rodent model of thoracic SCI where both cell types demonstrated clear tripotency and migration throughout the perilesional region. O-drNPCs also differentiated into significantly more mature oligodendrocytes, reduced the lesional volume, promoted white matter sparing, and produced enhanced long-term motor recovery. Furthermore, neither group showed evidence of tumor formation. This unique study provides key proof-of-concept data that hNPCs can be directly generated from somatic cells, the resultant product has hNPC functionality in vitro and in vivo, and transplantation of enriched O-drNPCs represents a viable approach to treat traumatic SCI.

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W-2118

AGE-RELATED DNA METHYLATION DYNAMICS IN IPSC-DERIVED AND DIRECTLY CONVERTED NSC

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Recent reports comparing induced neurons (iNs) with neurons differentiated from induced pluripotent stem cells (iPSCs) suggest that iNs, but not iPSC-derived neurons largely preserve age-associated traits. Hence, we became interested whether and to what extent directly converted stable induced neural stem cells (iNSCs) preserve epigenetic aging signatures. Employing restricted and integration-free expression of the transcription factors SOX2 and c-MYC we successfully converted adult human peripheral blood cells into clonally expandable iNSCs, which remain highly responsive to regional patterning cues and give rise to functional neurons, astrocytes and oligodendrocytes. We then studied the methylation status of CpG sites known to display differential methylation during aging, thereby enabling the determination of a 'DNA methylation (DNAm) age'. Using the Horvath model, DNAm ages of blood-derived iPSC and iPSC-derived neural progenitors were reset to a neonatal stage. In contrast, newly generated iNSCs exhibited DNAm ages situated between late childhood stage and the chronological age of the blood donors, suggesting a partial preservation of age-related DNAm signatures during direct cell fate conversion. Single cell-derived iNSC subclones showed variable DNAm ages, which might point to variable preservation and/or erosion of age signatures during generation and expansion of individual iNSCs. Interestingly, the DNAm age of iNSCs further decreased during extended passaging. Taken together, our findings argue against an extensive preservation of age-related epigenetic alterations in iNSCs, which makes this cell population an interesting resource for both, in vitro disease modeling and regenerative approaches.

W-2120

DIRECT REPROGRAMMING ADULT HUMAN FIBROBLASTS INTO INDUCED DOPAMINE PRECURSOR CELLS FOR PARKINSON'S DISEASE MODELLING

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To model a human neurodegenerative disease we need to generate live, but disease affected human neurons in the laboratory. Using cellular reprogramming to create induced pluripotent stem cells (iPSCs) from patients with Parkinson's disease means this is now possible. However, iPSCs are rejuvenated cells and neurons differentiated from them reflect a young phenotype, with some signs of aging reversed. To model an age-related disease we require a different method that retains age-related changes in the cells. Attempting to address this, we have developed a direct reprogramming technology that induces neural precursor cells (iNPs) directly from control adult human fibroblasts (aHDFs) and from Parkinson's disease patients carrying the LRRK2 mutation. We used transient transfection of stable, non-immunogenic modified RNA (SNIM®) for the neural stem cell master regulator gene SOX2, and dopaminergic lineage gene LMX1A, in a specialised reprogramming media under hypoxia to generate iNPs. Dopaminergic progenitors generated by SNIM-RNA reprogramming expressed ventral floorplate genes Sox2, Lmx1a, Lmx1b, Wnt1, Otx2 and FoxA2 by qPCR after 7 days of reprogramming, with sustained protein expression of SOX2, MASH1, NGN2, NURR1, and PITX3 observed in the following weeks. Differentiating dopamine neurons expressed TUJ1, AADC and TH and from 30 days began to express immature electrophysiological activity. Further, disease related changes were observed in Parkinson's disease iNPs during the reprogramming process, with altered SHH signalling and dopamine lineage genes identified through qPCR. Additional studies are being performed to fully mature the dopamine neurons to fire mature action potentials, and to identify further disease-related and age-related markers in diseased compared to control neurons. From these results will be able to identify molecular changes caused by the LRRK2 mutation, and provide a cellular platform for drug identification and screening for treatment for Parkinson's disease.

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W-2122

DIRECT REPROGRAMMING OF HUMAN FIBROBLASTS INTO DISTINCT STATES OF NAIVE PLURIPOTENCY IDENTIFIES KLF4 AS A CONDUIT FOR PRIMED TO NAIVE CONVERSION

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Recent studies have mainly focused on the derivation and characterization of naive human pluripotent stem cells (hPSCs), either by conversion from primed hPSCs or by direct derivation from human embryos rather than by somatic cell reprogramming. Moreover, naive hPSCs obtained in different naive-permissive media have been reported to have a different number of the characteristics that define a naive state and therefore cells from the inner cell mass (ICM). Here, we derived genetically matched human naive hiPSCs by direct reprogramming of fibroblasts as well as primed-to-naive conversion using different naive conditions (NHSM, RSeT, 5iLAF) and successfully reprogrammed fibroblasts to a naive state in t2iLGöY condition. Comprehensive characterization showed that naive hiPSCs obtained in these different conditions represent a spectrum of naive characteristics irrespective of whether they were derived by conversion or reprogramming. Importantly, only t2iLGöY hiPSCs displayed a similar transcriptome to human ICM cells, karyotypic stability and require re-priming for trilineage differentiation. Furthermore, our analyses identified KLF4 as a key reprogramming factor which enables conversion of primed hPSCs into naive t2iLGöY hPSCs. These findings underscore the role that reprogramming factors can play for the derivation of bona fide naive hPSCs and provide a molecular and functional reference for all the analyzed conditions, which will help accelerate the downstream applications of naive hPSCs.

W-2124

CLINICAL-GRADE IPSC GENERATED WITH A CGMP SENDAI VIRAL REPROGRAMMING KIT

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For the past decade, the induced pluripotent stem cell (iPSC) field has grown immensely, starting with basic research, and now moving more toward translational and clinical applications. As iPSC move towards the clinic, it is vital that the cells used are of high quality; free of reprogramming transgenes, free of adventitious agents, genetically stable, and functionally pluripotent. Traditional reprogramming workflows typically include one or more components of animal origin, such as fetal bovine serum (FBS) or bovine serum albumin (BSA), which can be sources of adventitious agents. This means that iPSC that are generated in a xeno-free, clinically relevant workflow will be much more readily applicable to clinical uses. Here, we show the generation of high quality iPSC with a cGMP-manufactured Sendai reprogramming kit, which is free of animal origin components. The kit was used to reprogram both blood-derived cells and skin fibroblasts, in conjunction with xeno-free cell culture and reprogramming workflows, starting from initial cell isolation, all the way through to iPSC expansion and banking. The iPSC generated with this method were shown to have a normal karyotype, were free of Sendai viral vectors, expressed the standard self-renewal markers, and demonstrated functional pluripotency for all three germ layers. The ability to create high quality iPSC in clinically relevant, xeno-free workflows, will allow researchers to more smoothly transition cells to clinical applications.

W-2126

PDX1-EXPESSION, RETINOIC ACID, LOW SERUM LEVELS AND NICOTINAMIDE ARE CRITICAL FOR INDUCING INSULIN EXPRESSION IN MOUSE ADIPOSE-DERIVED STEM CELLS

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Diabetes is a metabolic disease characterized by a deficiency of insulin relative to metabolic needs, leading to poor glycemic regulation. Management of insulin-dependent diabetes involves the administration of insulin with constant glucose monitoring, while transplant of donor or cadaveric pancreas/islets remains the only cure. However, the cure is limited by the scarcity of donors and tissues, hence stem cell-derived insulin-producing cells (IPCs) became regarded as a viable option. With the aim to establish a mouse IPC transplant model, we chose to reprogram adipose-derived stem cells (ADSCs) isolated from Mouse Insulin Promoter (MIP)-GFP mice. Previous studies showed that insulin expression could only be induced in ADSCs overexpressing pancreatic and duodenal homeobox 1 (PDX1; ADSCPDX1). In an extensive screen of published and in-house protocols, the successful protocols not only induced green fluorescence in ADSCPDX1 but also induced the expression of pancreatic hormones (insulin and glucagon). We identified 13 factors in these protocols, which are base medium, foetal bovine serum (FBS) concentration, retinoic acid (RA), activin A (ActA) concentration and treatment duration, exendin-4, glucagon-like peptide-1, trichostatin A, nicotinamide (NAM), fibroblast growth factor 1 and 2, epidermal growth factor and insulin-like growth factor. These were tested in various combinations in a series of fractional factorial design experiments. We found that FBS concentration, RA and NAM were significant contributors to the reprogramming of ADSCs and/or induction of insulin expression, while ActA's contribution was marginal. These 4 factors increased the expression of insulin and other pancreatic islet genes in the reprogrammed cells. To further improve the level of insulin induction and IPC reprogramming efficiency, we are currently investigating the impact of ADSC 'stemness' on IPC reprogramming efficiency. Hence, with the use of qPCR arrays to profile the expression of pluripotency/stem cell markers, we are studying pre-reprogramming treatment of ADSCs with hypoxia, chemical substitutes of the Yamanaka factors (e.g. RepSox for Sox2 and D4476 for Oct4), other epigenetic modifiers (e.g. 5-azacytidine, DZNep and parnate) and extracellular matrices (e.g. laminins, fibronectin and collagens).

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W-2128

ATRIAL NATRIURETIC PEPTIDE STIMULATED SKIN WOUND HEALING THROUGH ENHANCING PROPERTIES OF ENDOTHELIAL PROGENITOR CELLS IN MICE

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The atrial natriuretic peptide (ANP) is secreted by heart muscle cells and functions a powerful vasodilator. . We hypothesized that the ANP stimulates angiogenic properties of endothelial progenitor cells (EPCs). We measured the migration and the tube formation activities of EPCs after stimulation with ANP. Increasing dose of ANP enhanced migration and tube formation

of EPCs in vitro. In addition, ANP promoted the survival and reduced apoptosis of EPCs in the low-serum condition, which recapitulates the environment after transplantation to patients. Knockdown of natriuretic peptide receptor-1 (NPR-1) in EPCs with small interfering RNA (siRNA) significantly reduced ANP-induced EPC migration and tube formation. The angiogenic effect of ANP on EPCs was also abrogated by knockdown of NPR-1. These results demonstrate that ANP increases angiogenic properties of EPCs via NPR-1. For in vivo application, we evaluated the effect of ANP on skin wound healing in mice with single treatment or the combination treatment with EPCs. Mice were divided to 4 groups including HBSS, ANP, EPC, and ANP in combination with EPC. ANP treatment significantly accelerated wound closure in combination with EPCs in comparison with other groups. These results suggest that ANP enhances therapeutic potential of EPCs and may contribute to the development of a novel curative for accelerating wound healing.

W-2130

TARGETED ACTIVATION OF GENE EXPRESSION TO DRIVE AUDITORY HAIR CELL REPROGRAMMING

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Hearing loss is the most pervasive sensory deficit in humans, and the overwhelming majority of cases are caused by sensorineural hearing loss, defined by a deficit in function in the auditory hair cells, auditory nerves, or the ribbon synapses between them. Severe sensorineural hearing loss often entails loss of the auditory hair cells, and in mammals these hair cells are nonregenerative. Two recent advances using pluripotent stem cell models have demonstrated the generation of hair cells from pluripotent stem cell culture: (i) culturing with growth factors or drugs that recapitulate developmental cues over a period of weeks to generate in vitro the embryonic tissue layers that produce the otocyst with subsequent differentiation of the cells; or (ii) a reprogramming approach that uses ectopic expression of key transcription factors known to function in hair cell development. While these studies provide useful information on hair cell development in the embryo, the endogenous supporting cells of the cochlea have been shown to act as hair cell progenitors and represent a desirable target for gene therapy to regenerate hair cells. We are developing a method to deliver synthetic transcriptional activators based on CRISPR/Cas9 into an in vitro model of cochlear progenitor cells. This system enables targeted epigenetic modification and gene activation from endogenous gene promoters in an in vitro model of the inner ear. We have identified guide RNA sequences that can efficiently upregulate inner ear reprogramming genes in model cell lines. We have further found evidence that this system can be successfully delivered into cochlear progenitors. This method provides a platform for the activation of any unique gene promoter in an in vitro model of cochlear supporting cells, and the results will be directly applicable to future in vivo studies of cochlear development and transdifferentiation.

W-2132

DESTABILIZING ESTABLISHED TRANSCRIPTIONAL PROGRAMS TO ACCELERATE DIRECT CONVERSION, ENHANCE DISEASE MODELING

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The central hypothesis of transcription factor-mediated reprogramming relies on optimal cocktails of factors to redirect transcriptional networks and, thus, cellular identity. However competition between established and newly induced networks may limit the full adoption of the alternative identity during reprogramming, resulting in a spectrum of conversion. Although p53 inhibition has been shown to promote reprogramming, its mechanism of action remains unclear. Here, we show that due to transcriptional capacity constraints within cells, competition between the latent donor gene regulatory network (GRN) and the reprogramming factors limits conversion of fibroblasts into several cell types, including motor neurons. p53 inhibition enables converting cells to overcome this roadblock by efficiently silencing the donor GRN. Inhibition of the p53 DNA binding domain via p533DD extensively promotes direct conversion and morphological maturation across species and conversion protocols. Early in reprogramming p53DD promotes a division-dependent transcriptional shift downregulating fibroblast transcription factors. Using single-cell transcriptional profiling, we observe that reduction of the fibroblast GRN inversely correlated with morphological maturity of iMNs. In particular, Prrx1 expression was higher in cells with putative neuronal markers that failed to morphologically remodel. Overexpression of Prrx1 or dual blockade of miR-9 and miR-124 which are known to target Prrx1 inhibited p53DD-mediated increase in conversion and neuronal remodeling. Further, individual knockdown of fibroblast TFs (e.g. Prrx1, Tead1) increased conversion. Together these data suggest that destabilizing the established GRN is a critical step in direct conversion. Down regulation of the fibroblast GRN coincides with increases in TSS accessibility, and increased binding of the motor neuron reprogramming factors Isl1 and Lhx3 to their

target loci. iMNs converted with p53 inhibition (DD-iMNs) show enhanced electrophysiological maturation as well as enhanced selective vulnerability to disease-stimuli, suggesting these cells better model ALS disease processes in vitro.

TECHNOLOGIES FOR STEM CELL RESEARCH

W-2134

VALIDATION OF DEFINED FEEDER-FREE CULTURE SYSTEMS FOR THE MAINTENANCE OF EUTCD-COMPLIANT HUMAN EMBRYONIC STEM CELL LINES

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Validated protocols for the expansion of human embryonic stem cells (hESCs) within the UK Stem Cell Bank (UKSCB) include the use of inactivated feeders as well as the feeder-free alternative Matrigel-mTeSR™1 combination. These approaches, however, include the use of animal-derived products, undefined serum and other components that may pose a risk of microorganism transmission as well as carrying inherent batch-to-batch variability, which may ultimately result in undesirable changes in cellular function. These issues become even more pertinent when hESCs with potential clinical applications are being expanded, and therefore the development of a more standardised and well-defined approach to culturing hESCs with product safety and reliability in mind becomes crucial. Since the UKSCB is currently preparing to derive seed stocks of clinical-grade hESCs, a study to generate feeder-free protocols for the cultivation of EUTCD-compliant hESCs has been performed. During phase I of the study, a meta-analysis of published methods was conducted that identified 14 commercially available media and matrices combinations that were commonly used to culture hESCs. These combinations were then used to culture the well-characterised cell line H9 during phase II of the study (presented at ISSCR, 2015). Phase III is currently in progress whereupon the 3 most effective conditions from phase II are being assessed on several clinical grade hESCs based on criteria of phenotypic and genotypic stability, morphology and growth rate. As such, the cells maintained in each condition were morphologically examined for multiple serial passages and were assessed for differentiation potential by embryoid body formation, proliferative capacity, karyotypic stability and expression of known pluripotency markers by qPCR and multi-colour flow cytometry. The results from phase III of the study will be presented. Optimal media/matrix combinations identified in this study will subsequently be utilised for the maintenance of EUTCD compliant hESC lines, thus providing a better defined, feeder-free system for culturing clinical grade cell products.

W-2136

EVALUATION OF A TRANSCRIPTOMIC-BASED METHOD FOR COMPARING BIOEQUIVALENCE IN MODULATION OF GENE EXPRESSION OF BIOSIMILAR GCSF IN VITRO

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The objective of the present study was to evaluate the transcriptome of umbilical cord blood cells (UCB) exposed to a biosimilar and innovative G-CSF for the comparison of the molecular targets impacted by the two drugs, as well as to gain key gene information from the cellular response. Before signing the pregnancy consent form, 30 UCBs were obtained, to which ficoll-hypaque was performed, the mononuclear cells obtained were mixed to form a pool, these were subjected to 100 ng / ml GCSF. Three biological and technical replicates were performed using UCB cells (no stimulation). The cultivation took place 6 hours later wherby the mRNA was obtained, and gene expression analysis was performed using Agilent microarray. Data pre-processing and QC analysis was performed with R/Bioconductor LIMMA package. Differentially expressed genes by G-CSF treatment were identified using a Rank product test. Heatmap visualization and functional enrichment analysis were performed with MultiExperiment Viewer software and Cytoscape InnateDB respectivelly. The G-CSF is both biosimilar and innovative inducing the overexpression of 299 genes in cells obtained from UCB. Functional enrichment analysis with KeggPathway revealed 10 signaling pathways enriched with statistical significance: chemokine signaling pathway p 0.0205, signaling pathway PI3K-Akt p 0.01214; Notch signaling pathway p 0.01170; Hippo signaling pathway p 0.05441; cell cycle p 0.00210; assembly of focal adhesions p 0.01084; proliferation of stem cells p 0.03999 and cell migration p 0.045933.. These transcription factors are associated with Wnt signaling pathways (CTNNB1, EP300, TP53) p = 0.000936, MAPK signaling pathway (FOS, NFKB1, TP53) p = 0.00393, Notch pathway (EP300, NOTCH1) p = 0.00412, B-cell receptor signaling pathway (FOS, NFKB1) p = 0.0061, Apoptosis (NFKB1, TP53) p = 0.0078, T cell receptor signaling pathway (FOS, NFKB1) p = 0.00923, Toll receptor signaling pathway -like (FOS, NFKB1) p = 0.00949, signaling pathway HIF-1 (EP300, NFKB1) p = 0.00957, TNF (FOS,

NFKB1) signaling pathway p = 0.00983 and cell cycle (EP300, TP53) p = 0.0121. Stimulation with both drugs suggests increased pathways associated with innate immunity and cell migration

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W-2138

FULL-TIME PHYSIOXIC CONTROL MAXIMIZES HUMAN MSC EXPANSION AT THE INDIVIDUAL CELL AND POPULATION-WIDE LEVELS

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Mesenchymal Stem/Stromal Cells (MSC), derived from tissues that normally experience low oxygen levels, are of intense interest for a wide variety of clinical applications including cartilage, skin and bone repair. Researchers often incubate MSC at physiologic oxygen (physioxic) conditions. However, when handled using conventional room air BSCs, these MSC experience highly variable supraphysiologic oxygen conditions and suffer oxidative stress. Using the Xvivo System platform, we can control all temperature and gas levels full-time, during all cell handling steps as well as incubation steps. With robustly controlled conditions, more refined optimization of oxygen levels is possible. Our null hypothesis was that cell growth characteristics of human bone marrow MSC exposed to constant supraphysiologic oxygen conditions would not be different from those exposed to full-time physioxia. Human bone marrow MSC cultures were divided and cultured at 5% CO2 and 1%, 2%, 3%, 5%, or 18% (standard CO2 incubator) O2. The cell processing chamber atmosphere was set to match the incubation conditions for each culture, so each MSC culture was in constant conditions at all times. All solutions were pre-incubated to the appropriate oxygen levels before use. Standard trypan blue counting was used to estimate cell culture densities at each passage and standard colony forming assays were used to assess clonogenicity. Higher cumulative cell yields and faster cell growth were seen when cells were incubated and handled at 2-5% O2. On an individual cell basis, MSC were also more likely to retain clonogenic capacity when incubated at these oxygen levels. At the population level, the MSC produced more passages before senescence when maintained below 5% O2. This was not an obvious cytotoxic effect, but an effect upon the number of cells in each generation that remain in the cell cycle. We conclude that constant control of oxygen levels below 5% O2 can help extend MSC growth beyond that obtained in room air culture.

W-2140

NEW TOOLS FOR IMPROVING THE GENOME EDITING WORKFLOW IN HUMAN INDUCED PLURIPOTENT STEM CELL APPLICATIONS

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Induced pluripotent stem cells (iPSC) and their differentiated progeny provide useful tools for drug discovery and promise to provide more predictive results from assays that translate to pre-clinical and clinical outcomes with greater fidelity than current non-human models. Disease-specific iPSCs can be derived directly from patients with known disease phenotypes or can be mimicked by introducing known mutations into the iPSC genome through CRISPR/Cas9 or TALEN systems. The genome editing process in iPSCs - especially homology directed repair (HDR) and single cell cloning - remains inefficient and more optimal workflows are needed to improve the application of genome editing to iPSC disease modeling. Work reported previously demonstrates that Cas9 protein and in vitro transcribed gRNA delivered through either electroporation or lipofection yields high editing efficiency in human iPSC. Here, we show that a stably expressing Cas9 human iPSC line and its differentiated progeny can be useful for genome editing-based applications. Stable expression of Cas9 yields up to 75% indels and up to 40% efficiency in HDR-mediated SNP corrections in iPSC. Moreover, high indel rates can be achieved when gRNAs are delivered to differentiated cells directly. However, while typically only optimization of editing tools is considered essential for success, the importance of human iPSC culture conditions during genome editing is often underappreciated. In particular, iPSC survival after gRNA/Cas9 delivery and isolation of single cells for derivation of homogenous clones has proven challenging and limits the ability to easily isolate homogeneous clones from genome edited iPSCs. We demonstrate that, through the combined use of the matrix protein Laminin 521 and the novel culture medium StemFlex, cell survival is greatly improved during the genome editing workflow. Most importantly, we establish that these reagents are key to supporting single cell isolation by automated cell sorting from genome edited iPSCs, methods that are also applicable to transgenic iPSCs generated through lentiviral or plasmid based methods. These tools and methods thus contribute to improved success in the derivation of homogenous genome edited human iPSC clones and furthermore provide novel alternatives to study disease-causing mutations in vitro.

W-2142

STEM CELL COMMONS: A DATA MANAGEMENT, ANALYSIS, AND VISUALIZATION PLATFORM FOR HIGH-THROUGHPUT SEQUENCING EXPERIMENTS

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The number of high-throughput sequencing applications is ever-increasing which, along with consistently plummeting costs, has significantly increased the accessibility of this technology. However, progress has comparatively lagged behind in making bioinformatics solutions available to wet lab biologists that allow them to manage and explore their data as straightforwardly as they can now produce it. While some data analyses will require the expertise of bioinformatics specialists, others that now have reasonably standardized workflows should be able to be largely directed by the wet lab biologists who are frequently best equipped to guide the data exploration trajectory. Consequently, we have developed the Stem Cell Commons platform for the Harvard Stem Cell Institute to provide non-specialists with solutions for storing data, executing popular analysis workflows (e.g. RNA-seq, ChIP-seq), and visualizing results. Stem Cell Commons unifies its data repository function with its analysis workflows, which are executed via the Galaxy workbench, allowing users to easily transition between data management and processing. Also, the platform integrates rich metadata annotations as well as data provenance information, thus facilitating reproducible analyses. Furthermore, users can share uploaded data with collaborators, situating Stem Cell Commons as a central repository where well-described data can be reliably stored and distributed across laboratories. Stem Cell Commons can be accessed at https://beta.stemcellcommons.org, and all data and tools are open source and freely available. Our goal is that this platform encourages more stem cell biologists to pursue high-throughput sequencing experiments and particularly enables them to independently explore their data to foster new insights.

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W-2144

THE HUMAN CELL UNIVERSE: DEVELOPMENT OF INDUSTRIAL-GRADE PROTOCOLS FOR SPECIALIZED CELLS USING SYSTEMS DEVELOPMENTAL BIOLOGY

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Current approaches generally fail to provide the needed process understanding for scalable and robust biologic manufacturing. Typical laboratory investigations are unable to extract statistically-defined identification of critical process parameters, yet are able to publish in leading journals. While clinical testing of living human cells in man have commenced, there remain many challenges to be overcome, especially understanding how to achieve maximal potency, and maximal specificity as it relates to the disease in which such cells are tested. The paradigm for R&D efforts towards cell therapy development is one of single pathway interrogation which is neither ideal nor representative of the complexity of the living system. Practically, the work is performed by hand; experimental designs are limited by the human mind; and thus the experimental space that is tested remains narrow and incapable of penetrating the interaction complexity of a given developmental process. Consequently, we currently lack robust production methods for the larger complement of specialized human cells. We here describe a technology platform that rests on high-dimensionality testing methods towards making specialized human cells from stem cell sources. During design of this platform, we considered that cells process information in an ongoing combinatorial manner where many environmental cues give a cell a combined set of instructions to remain as it is, or possibly change. To achieve a much increased power of experimentation, we used industrial software and integrated this with liquid handling robotic hardware and then developed a set of principles to execute arguably the deepest experimental designs performed in developmental biology to date. As a result, we are able to solve recalcitrant combinatorial problems in cell culture. In technical terms, the method is based on Design-of-Experiments (DoE) mathematics, and is compliant with Quality-by-Design principles currently adhered to by the pharmaceutical industry yet not expected within the regenerative medicine arena. As an integrated project in the newly established biofabrication institute ARMI, (Advanced Regenerative Manufacturing Institute) we are applying this technology to deliver a industrial-grade protocol catalogue of >50 different human specialized cells.

Funding Source: Ohio Third Frontier, and Department of Defense

W-2146

TARGETED INSERTION OF A LARGE XIST TRANSGENE TO "SILENCE TRISOMY" IN HUMAN AND MOUSE STEM CELL MODELS OF DOWN SYNDROME

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Down syndrome is the leading genetic cause of intellectual disabilities in children, however it remains unknown how many of the ~400 genes on chromosome 21 even have an impact when expressed just 50% more, impeding any drug development and precluding any possibility of genetic therapies. Our lab recently showed that the natural X-chromosome dosage compensation mechanism, controlled by X-linked XIST gene, can be used to silence a trisomic chromosome by targeted insertion of a large (~20 kb) XIST transgene. XIST expression on the trisomic chromosome improves proliferation and neural stem cell formation of Down syndrome iPS cells. The inducible "trisomy silencing" system opens a unique opportunity to identify molecular networks driving difference aspects of cell pathologies, and a tractable way to distinguish primary versus down-stream effects of trisomy 21 expression. This strategy has identified new candidate pathways and non-chr21 genes potentially important to the underlying biology of Down syndrome, which will be discussed. In parallel, we have made progress towards applying the chromosome silencing strategy to "correct" a trisomic mouse model of Down syndrome. We have tested different gene editing technologies (ZFNs and Cas9/CRISPR) to target the large Xist transgene in mouse ES cells. Using Cas9/CRISPR, we have successfully integrated a large mouse Xist transgene into the trisomic chromosome in Ts65Dn mouse ES cells with 36% targeting efficiency. Our preliminary data have demonstrated chromosome silencing on the targeted chromosome. Generation of trisomy correction mouse model is in progress.

W-2148

CLONAL SELECTION AND ASYMMETRIC DISTRIBUTION OF HUMAN LEUKEMIA IN MURINE XENOGRAFTS REVEALED BY CELLULAR BARCODING

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Genetic and phenotypic heterogeneity of human leukemia is thought to drive leukemia progression through a Darwinian process of selection and evolution of increasingly malignant clones. However, the lack of markers to uniquely identify leukemia stem cells (LSC) precludes high resolution tracing of LSCs and their clonal offspring. Here, we employ cellular barcoding to analyze the clonal behavior of patient-derived LSCs in murine xenografts. Using a leukemic cell line and diagnostic bone marrow cells from six patients with B-progenitor cell acute lymphoblastic leukemia (B-ALL), we demonstrate that the murine environment provides a selective bottleneck, only allowing engraftment of a subset of LSC clones. Nonetheless, patient-derived xenografts were highly polyclonal, consisting of tens to hundreds of LSC clones. The number of LSC was stable within xenografts but strongly reduced upon serial transplantation. In serial xenografts, clonal composition was highly similar between recipients of the same donor and reflected donor clonality, supporting a deterministic, clone-size based model for clonal selection. Finally, quantitative analysis of clonal abundance in several anatomic sites identified two types of asymmetry. First, clones were asymmetrically distributed between different bones. Second, clonal composition in the skeleton significantly differed from extramedullary sites, showing similar numbers but different clone sizes. Cellular barcoding method can be compared with other clone-tracking techniques in vitro and in vivo. Although its major limitation is in vitro labelling protocol, it remains superior to other currently used methods regarding unique cell labelling and detection. Altogether, this study shows that cellular barcoding and xenotransplantation provides a useful model to study the behavior of patient-derived LSC clones, which provides insights relevant for experimental studies on cancer stem cells and for clinical protocols for the diagnosis and treatment of leukemia.

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W-2150

AUTOMATED IMAGING, ANALYSIS AND SELECTION TOOLS TO CHARACTERIZE HETEROGENEOUS STEM/PROGENITOR CELLS AND ISOLATE CELLS WITH DESIRED BIOACTIVITY

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The promise of cell based therapies demand development of rapid, reliable, reproducible tools and techniques that can: i) capture large field of view (LFOV) images with sufficient quality to enable quantitative analysis ii) define and quantify the critical quality attributes (CQA) of stem and progenitor cells with respect to morphology and molecular markers iii) correlate these CQA with their long term biological performance and iv) specifically isolate separate desired cells/colonies from undesired cells to optimize reproducible biological performance. These tools and techniques are necessary to understand the composition, phenotype and range of biological potential among stem/progenitor cells in native human tissues, and to optimized cell sourcing protocols for tissue engineering applications. Cell X is a robust robotic system that allows automation of these process steps. LFOV images can be acquired from any standard 6, 12, 24 or 96 well plate at 1µ/pixel resolution with acquisition times of 1-8 minutes, using brightfield or fluorescence. Time-lapse imaging is also supported. Time-lapse imaging enables founding cell attributes and early colony performance attributes to be linked to the long-term future performance. Colonyze is a software platform that enables Automated Multimodal Large field of view Image Analysis (AM-LIA) based on proliferation, migration, auto fluorescence, cell surface markers, and cell and colony morphology. The principles and nomenclature enabling this approach are outlined in ASTM Standard F2944-12. The integrated Cell X and Colonyze platform enables automated iterative processes for high speed "picking" or "weeding" of desired or undesired cells/colonies based on defined and reproducible criteria, eliminating manual processing steps.. The Cell X provides unprecedented opportunities in the analysis and management of heterogeneous cell populations when used for selection of starting material or for highly controlled and documented "in process" refinement steps focusing on CQAs.

W-2152

LONG-TERM COMPARISON OF TRANSACTIVATORS FOR INDUCIBLE TRANSGENE EXPRESSION IN HUMAN IPSC-DERIVED NEURAL STEM CELLS

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Several doxycycline-responsive transactivators have been reported to transiently provide non-leaky and robust transgene expression in vitro and in vivo in response to doxycycline. These findings provide promise for the study of developmental pathways where genes are transiently expressed and repressed during stem cell differentiation and organogenesis. Additionally, preclinical studies have benefited from this technology as stem cells can be transplanted and allowed to engraft and propagate before being molecularly differentiated to intricate cell types such as projection neurons. It also allows for the temporally regulated secretion of neuroprotective growth factors for preserving degenerating neurons in transplanted cells for neurodegenerative diseases such as Parkinson's disease and amyotrophic lateral sclerosis (ALS). This type of externally regulated transgene expression may limit the potential adverse effects associated with constitutive transgene expression. However, the use of doxycycline-dependent transactivators to regulate gene expression in clinical settings necessitates an assessment of their long-term safety and efficacy. Specifically, there have been reports of engineered transactivators exhibiting transcriptional squelching and other toxic effects when expressed for an extended period of time—limiting their potential use in patients. Here, we compare the long-term ability of the VP48, p65, RTA, and VPR transactivators to induce or repress transgene expression in induced pluripotent stem cell (iPSC)-derived neural stem cells. Furthermore, we employ a novel dual recombinase-based methodology for rapidly inserting single-copies of genetic elements into the AAVS1 safe landing site. Our results provide a quantitative long-term comparison of the efficacy and toxicity of these transactivators when used to induce gene expression in iPSCs at the single-copy level. These findings provide a much-needed investigation of short-term vs long-term robustness and toxicity for the use of doxycycline-inducible gene activation.

Funding Source: California Institute for Regenerative Medicine

W-2154

PROVIDING AND DEFINING A SOLUTION FOR CELL THERAPY SAFETY

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Cell therapies are currently being explored to treat degenerative diseases; however, safety concerns such as potential tumourigenicity have limited their utility and implementation. Here we introduce a concept and an associated genome editing strategy that addresses the issue of cell therapy safety. To ensure the operation of an inducible suicide system in potentially harmful cells, we transcriptionally linked a negative selectable transgene to a cell division essential locus (CDEL) with a viral 2A sequence. Our prototype suicide gene and CDEL is HSV-TK, and CDK1, respectively. First, we generated mouse and human ESC lines with the described heterozygous modification. Our results show that we can eliminate proliferating cells both in vitro and in vivo with the treatment of the prodrug ganciclovir (GCV). The elimination of these proliferating cells can efficiently stop the growth of teratomas generated by these ESCs, and renders this tissue dormant for up to one year. Despite the teratoma dormancy post-GCV treatment, we are able to detect GCV "escapees" among heterozygous cells, and found that the majority of them (6/8) were the result of loss-of-heterozygosity (LOH). To avoid the loss of the suicide gene by LOH, we generated cell lines with a homozygous CDK1 modification. Since we were unable to identify escapees in these lines, we used mathematical modeling to quantitate the risk of generating a GCV-resistant escapee. Furthermore, we demonstrated that the homozygous modification of CDK1does not compromise the developmental potential of the cells. Mice that are homozygous for the modified allele are viable, and modified hESC lines are able to differentiate into a range of therapeutically relevant cell types. Lastly, we illustrate that GCV spares quiescent therapeutic cells. We are confident that the strategies presented and characterized will provide solutions for cell therapy safety and will enable the use of a large number of in vitro generated, and expanded cells.

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W-2156

ADVANCED CELL CULTURE TECHNIQUES TO MAINTAIN THE NATIVE STRUCTURE-FUNCTION RELATIONSHIP OF PLURIPOTENT STEM CELLS

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Pluripotent stem cells (PSCs) are ideal sources of cells for tissue engineering due to their unlimited capacity to self-renew and differentiate into lineages of all three germ layers. PSCs are routinely grown in vitro as monolayers on flat tissue culture plastic. These methods limit cell-cell and cell-matrix interactions, resulting in changes to the cellular cytoskeletal architecture, reduced maintenance of cell renewal, and limited differentiation potency through altered mechanotransduction. We hypothesise that such changes in cell architecture impact on the ability of PSCs to grow and differentiate to form complex tissue-like structures. In this work we present a novel approach that provides a three dimensional (3D) microenvironment to maintain the natural 3D architecture of PSCs. We use a synthetic scaffold that provides a physical 3D space that maintains the native cytoskeletal structure to "3D prime" cells for enhanced function in subsequent work flows. Alvetex® Strata is a scaffold membrane that has previously been optimised for the growth of human embryonal carcinoma (EC) cells. This 10 day priming period results in marked re-arrangement of primary structural cytosketelal elements, such as the F-actin cytoskeleton. Quantified expression of pluripotency markers are maintained and in some cases enhanced in 3D-primed PSC populations. 3D-primed cells also show enhanced aggregation and differentiation, for example, towards neuronal lineages. This enhancement of functionality provides encouraging evidence that the process can also be applied to mouse embryonic stem cell (mESC) populations. As such mESCs were optimised for culture on Alvetex® Strata. Preliminary work shows that culture upon this substrate is possible without gelatin coating conventionally required to maintain differentiation potential and self-renewal. In addition, mESCs show differences in morphology in line with that demonstrated with human EC cells. Future work will investigate the developmental potency of 3D-primed PSCs. Overall the 3D-priming of PSCs using Alvetex® Strata may provide the opportunity to maintain 3D cell architecture resulting in enhanced PSC maintenance and differentiation in vitro. Such technology may be used to create populations of PSCs with greater developmental potency for a range of applications.

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W-2160

NOVEL TRANSPLANTATION MODALITIES FOR GENERATING TRANSCRIPTIONALLY DEPENDABLE NEW MICROGLIA FROM HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Recent evidences indicate that hematopoietic stem and progenitor cells (HSPCs) can serve as vehicles for therapeutic molecule delivery to the brain by contributing to the turnover of resident myeloid cell populations. However, the nature of the cells reconstituted after transplantation and whether they could comprise bona fide microglia remain to be assessed. Moreover, the still limited efficiency of the process and its long timing advocates for novel strategies to enhance the potential associated clinical benefit for patients. In this work, we firstly demonstrate that HSPC transplantation can generate transcriptionally-dependable new microglia through a stepwise process reminiscent of physiological post-natal microglia maturation and new forming microglia like cells are transcriptionally distinct from bone marrow resident or circulating myeloid cells. We also unambiguously identified in the long-term hematopoietic stem cell compartment the cell fraction within murine and human HSPCs that mostly retains the ability to reconstitute microglia upon transplantation, likely favored in their trafficking to the brain by CXCR4 expression. Finally, generation of microglia-like cells of donor origin was firstly obtained also upon intra-cerebral ventricular delivery of HSPCs and this novel delivery route is associated to a clinically relevant faster and more widespread microglia replacement compared to systemic injection, confirming our original hypothesis that microgliosis could derive from an independent seeding of the brain by the intra-venously transplanted HSPCs. Overall, this work supports the relevance and feasibility of employing HSPCs for exerting therapeutic effects in the central nervous system (CNS), and identifies novel modalities, based on selection of populations to be transplanted and use of innovative transplant routes, for increasing the actual contribution of the transplanted cells to microgliosis and their therapeutic activity.

W-2162

EFFICIENT ESTABLISHMENT AND LONG-TERM MAINTENANCE OF 3-DIMENSIONAL HUMAN SMALL INTESTINAL AND COLONIC ORGANOIDS USING A NOVEL INTESTICULT™ ORGANOID GROWTH MEDIUM (HUMAN)

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Intestinal organoids are a valuable model for studying epithelial stem cell biology as well as for investigating the structural and functional mechanisms of the mammalian intestine. Recently we released IntestiCult™ Organoid Growth Medium - Mouse (OGM; STEMCELL), a novel medium for the establishment and long-term maintenance of mouse intestinal organoid cultures. This medium has helped eliminate much of the variability inherent to mouse intestinal organoid cultures and allowed for greater standardization. To develop a formulation of IntestiCult™ OGM for the growth of human intestinal organoids, intestinal crypts were isolated by incubating human small intestine and colon tissue samples with Gentle Cell Dissociation Reagent (GCDR) for 30 minutes at 4^{0}C with gentle agitation. The liberated crypts were then plated in a Corning® Matrigel® dome, flooded with IntestiCult™ OGM (Human) and cultured at 37^{0}C with 3 medium changes per week. Spherical organoid structures could be identified within 2 days of initial seeding. The mature organoids were expanded by harvesting, dissociated by manual agitation and 10 min GCDR incubation and re-plated at a 1:4 split ratio every 10-12 days over 15 passages. Organoids analyzed by immunohistochemical and qRT-PCR analyses for intestinal stem (Lgr5 and axin2), paneth (lysozyme), enteroendocrine (chgA), goblet (muc2) and enterocyte (villin) cell marker expression, revealed that organoids were comprised of both stem and differentiated cell types, demonstrating cultured human organoids closely resembled human intestine structure. We further validated these organoids are amenable to functional assays by treating 10 day-old colonic organoids with either 5μM Forskolin or DMSO and measuring organoid size increase as an indicator of swelling, similar to mutant detection assays performed for Cystic Fibrosis Transmembrane Receptor (CFTR). Organoids treated with Forskolin increased by 34.3 ± 4% (mean ± SD; n>100 organoids measured) within 120 minutes while the DMSO treated organoids had no significant increase, confirming that organoids cultured in IntestiCult™ OGM (Human) are functional. In summary, IntestiCult™ OGM for human intestinal cultures provides researchers with

a physiologically relevant model system for studying intestinal function and stem cell biology ex vivo.

W-2164

NETWORK FORMATION IN MOUSE IPSC-DERIVED EXCITATORY/INHIBITORY NEURONS AND PRIMARY CORTICAL NEURONS IN A MICROFABRICATED CO-CULTURE DEVICE

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Excitatory/inhibitory (E/I) imbalance in cerebral cortex can cause central nervous system disorders, such as schizophrenia and epilepsy. Although pathogenic factors disrupt E/I balance in restricted area, the imbalance leads to dysfunction in broad area. In order to reveal pathogenic mechanisms, it is needed to study how E/I imbalance in a sub-network causes abnormal activity in the surrounding network. However, there is a lack of useful tools for elucidating activity patterns in neuronal networks with heterogeneous E/I balance. Here, we aimed to develop an in vitro experimental system for studying synchronized activity of neuronal networks composed of two sub-networks with different E/I balances. First, activity was recorded from neuronal networks containing excitatory or inhibitory neuron-rich populations with microelectrode arrays (MEAs). For obtaining excitatory/inhibitory-rich neuronal populations, mouse induced pluripotent stem cells (miPSCs) were selectively induced to differentiate into glutamatergic/GABAergic neurons. The excitatory/inhibitory-rich neuronal populations showed different activity patterns. Synchronized activity was more dominant in the excitatory population than in the inhibitory one. Next, miPSC-derived neurons were co-cultured with mouse primary cortical neurons in a co-culture device. Our device has two culture compartments interconnected via 30 microtunnels. The device was mounted on an MEA for activity recording. Cortical neurons were seeded into one compartment, and miPSC-derived neurons into the other. They formed sub-networks in each compartment and functionally connected via microtunnels. Synchronized bursting between primary and miPSC-derived networks was recorded under both excitatory- and inhibitory-rich conditions. These results indicate that our device is feasible to evaluate synchronized activity generated from neuronal networks composed of sub-networks with different E/I balances.

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W-2166

CRYOPAUSE: A NEW METHOD TO IMMEDIATELY INITIATE EXPERIMENTS AFTER CRYOPRESERVATION OF PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (PSCs) provide an unlimited cell source for cell replacement therapies and a new tool for disease modeling applications. Despite their enormous power, technical aspects of stem cell culture have led to limited reproducibility. We describe here a simple modification of PSC workflow that eliminates a major variable for nearly all PSC experiments: the quality and quantity of the PSC starting material. Most labs serially passage PSCs and perform experiments when needed; consequently, cells used in supposedly comparable experiments can widely vary in passage number and quality. Additionally, the "just in time" nature of these experiments means that quality control rarely happens before use, potentially leading to compromised PSC quality, sterility and genetic integrity. Our method, called CryoPause, banks PSCs as a single cell suspension that can be thawed and plated directly into a differentiation or transfected out of the vial. Each CryoPause bank only needs to be quality tested once to validate hundreds of millions of cells that may be used for experiments at any later time from an identical starting PSC population. CryoPause also allows geographically separated laboratories to experiment on an identical starting PSC population. CryoPause increases reproducibility in both disease modeling and cell therapy applications, and eliminates many variables normally present in traditional stem cell culture.

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W-2168

SUSPENSION CULTURES OF HUMAN PLURIPOTENT STEM CELLS AS AGGREGATES IN ORBITAL SHAKE FLASKS

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mTeSR™3D is a defined medium based on mTeSR™1 optimized for fed-batch suspension culture of human

pluripotent stem cell (hPSC) aggregates. 3D suspension culture of hPSCs enables production of cell numbers not practical in 2D adherent cultures. However, transitioning cells from 2D culture to aggregate culture poses challenges in maintaining growth and pluripotency as volumes increase. Robust protocols are needed to transition cells to suspension culture and then scale-up volumes to obtain the required cell numbers. hPSC aggregate culture in low adherence 6-well plates on orbital shakers enables cell seeding density, suspension adaptation and passaging to be readily optimized. Here we present studies aimed at scaling 6-well plate cultures to larger orbital shaker-bottle cultures. Ideally, aggregate culture performance in 2 mL orbital plate cultures should be predictive of cultures in 10 - 100 mL shaker bottle cultures. Only a narrow range of stirring rates support optimal hPSC aggregate growth in suspension cultures. We found that an optimal orbital speed of 70 rpm (1.9 cm orbit) for 6-well cultures was also the minimum speed required to adequately suspend aggregates. The experimentally determined optimum agrees closely with theoretical minimum threshold mixing rates required to achieve bottom reaching vortices in a cylindrical orbitally shaken vessel. Experiments were conducted to determine the culture volume range over which optimal conditions identified in 6-well cultures could be scaled to larger volume orbital shaker cultures. A variety of up-scaling approaches based on liquid volume, mixing rpm, and vessel geometry were evaluated in cultures of hPSCs (H7, H9) or induced hPSCs (WLS-1C, STiPS-M001). Conditions were tested in cylindrical and Erlenmeyer-type flasks. Scaling the orbital speed based on maintaining equivalent hydrodynamics gave consistent performance as culture volumes increased. Thus, appropriate mixing speeds at different culture volumes can be estimated knowing liquid height, flask diameter and shaker platform orbit diameter. Expansion rates of hPSCs cultured as aggregates in shake flasks ranged from 1.3 to 1.6 fold per day (dependent on cell line) with maintenance of pluripotency marker expression (Oct-4, Tra-1-60) and trilineage potential.

W-2170

RNA REPLICON PLATFORM TO ENABLE LONG-LASTING TRANSIENT EXPRESSION IN PRIMARY AND STEM CELLS

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RNA replicons are an emerging platform for delivering complex genetic content into mammalian cells. Replicons are synthetic mRNA molecules that include viral nonstructural proteins (nsP1-4) from Alpha viruses such as Semliki Forest Virus and Venezuelan Equine Encephalitis Virus (VEE). Replicons can be transfected into cells and can self-amplify by virtue of a self-encoded RNA-dependent RNA polymerase. These molecules provide long-lasting, high-level gene expression from a few initial RNA molecules, making their use ideal for gene transfer applications needing sustained expression. Current mRNA generation kits are suited for smaller (5kb or less) transcripts sizes and thus are not ideal for RNA replicons that are typically over 10 kb. In addition, the 5' end is less amenable to capping, necessitating enrichment and quantification of the capped functional replicon. Here we propose construction of a robust self-replicating vector platform with intrinsic structures that enable enrichment of functional mRNA. As a proof of principle we constructed a vector encoding self-replicating EmGFP and sustained GFP expression was verified in human dermal fibroblast and resting as well as activated T cells. The utility of this platform was further extended in other primary cell types and stem cells as well. Applications that might benefit from replicon use include RNA Chimeric Antigen Receptor – T cell immunotherapy, iPS cell reprograming strategies using multiple factors to modulate cell fate, and engineering via synthetic gene circuits.

W-2172

A NOVEL METHOD ENABLING THE SIMULTANEOUS DERIVATION OF DISEASE-RELEVANT POINT-MUTANTS AND CONCORDANT ISOGENIC CLONES FROM HUMAN IPS CELLS

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Gene-edited induced pluripotent stem (iPS) cells have established powerful human disease models in patient-specific or standardized genetic backgrounds. Towards this end, classic gene targeting with antibiotic selection markers remains the most reliable method for enriching targeted cells containing engineered mutations. Nevertheless, integrated selection markers obstruct subtle genetic modification and "scarless" transgene-free gene editing. Here, we present a novel method to remove selection markers by harnessing the endogenous microhomology-mediated end joining (MMEJ) DNA repair pathway. To do so, typical positive-selection markers are flanked by a short duplication of the target locus resulting in tandem repeats (microhomology). To engage MMEJ, DSBs are formed by CRISPR/Cas9 nuclease cleavage at standardized protospacer sequences nested between the cassette and engineered microhomologies. Moreover, using microhomology with a unilateral point mutation configuration, both mutant and normal isogenic clones which have undergone comparable cell culture manipulations can be derived

simultaneously, presenting a new paradigm for deriving appropriately matched isogenic controls. The efficiency and fidelity of our method is demonstrated by the deposition of patient-specific point mutations at various loci implicated in human metabolic disease, such that the engineered iPS cell clones elicit disease-relevant molecular phenotypes.

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W-2174

DEVELOPMENT OF A CELL THERAPY PAT STRATEGY BASED ON MULTI-PARAMETRIC PRODUCT CHARACTERISATION

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Successful industrialisation of cell manufacturing requires scaled-up/out culture platforms operated by quality-driven in-process controls. The utilisation of Process Analytical Technologies (PAT's) are positioned at the core of such in/at line controls, however their practical implementation is currently impaired by a range of challenges. Amongst these obstacles, the relationship between PAT's measurements and the Critical Quality Attributes (CQA's) of the cell products prove particularly difficult to establish. We implemented a three stages methodology that bridges the gap between inferential PAT's measurements and product specific characteristics, applied to the manufacture of induced pluripotent stem cells. In the first stage, high-throughput screening technologies, predominantly flow cytometry and RT-qPCR dynamic arrays, are used to screen hundreds of product specific and generic markers. Cellular metabolism and stress markers are also included in these panels. Cells grown in defined stress conditions, i.e. missing growth factors or high cell density, are compared to cell manufactured through a reference control process in which CQA's are maintained. Marker expression is analysed using differential and correlation techniques, from which a reduced panel of identity and quality markers modulated by culture conditions is identified. In the second stage, this panel is further augmented with hundreds of parameters obtained using PAT's such as LC-MS, metabolite analysers, protein arrays and quantitative imaging. Several manufacturing processes are run according to a Design-of-Experiment approach, acquiring all measurements simultaneously at regular intervals. In stage 3, the resulting large multi-parametric datasets are processed using co-expression network analysis, clustering correlated and connected markers. A data reduction scheme then allows the efficient selection of a bespoke subset of product specific markers, whose modulation is directly linked to manufacturing parameters. These markers can therefore be used for in-process monitoring and control. Such strategy is amenable to the selection of robust PAT's measurements, has applications across a wide range of cell and gene therapies, and has the potential to ensure controllable and repeatable manufacture.

THURSDAY, JUNE 15, 2017

POSTER SESSION II-ODD
18:00 – 19:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

T-1001

MESENCHYMAL STROMAL CELLS DELIVERED INTRAMUSCULARLY IN MICE REDUCE REMOTE INFLAMMATION

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Mesenchymal stromal cells (MSCs) have shown to respond to remote inflammation through secretion of soluble factors. MSCs are commonly administered intravenously (IV) which leads to rapid entrapment in the lungs and subsequent dissipation. However we, and others, have shown that intramuscular (IM) delivery results in increased MSC dwell time, which could result in longer-term secretion of anti-inflammatory mediators and thus sustained modulation of the inflammatory milieu. We hypothesized that, IM transplanted MSCs could effectively downregulate a remote inflammation. We used the hind paw model of inflammation, and unilaterally injected 9-week old CD1 mice with 1% w/v carrageenan. At the peak of inflammation (4 hours post induction), we delivered saline (control), human bone

marrow derived MSCs (hbmMSCs), human umbilical cord perivascular cells (HUCPVCs), or mouse bone marrow derived MSCs (mbmMSCs)—nominal dose 1.3x106 cells—into the contralateral quadriceps (n=6-7/group). Inflammatory modulation was monitored by circumferential paw measurements and paw tissue myeloperoxidase (MPO) activity at sacrifice 4, 24, and 48 hours post induction. In addition, serum, muscle, and organs were collected for assessment of anti-inflammatory and pro-inflammatory mediators, as well as the biodistribution of male mbmMSCs and human sequences within organs and inflamed paw. In all groups, including controls, the inflammatory level decreased from 24 to 48 hours. However, both human MSC populations showed significant MPO downregulation; hbmMSC (~1.75 folds by 24 hours and further ~2.59 folds decrease by 48 hours) and HUCPVCs (~3.72 folds by 24 hours and further ~5.52 folds decrease by 48 hours) compared to controls. mbmMSCs showed no such change. Delta paw circumference showed decreases in all MSC groups: mbmMSCs ~1.14 folds decrease by 24 hours and ~1.46 folds decrease by 48 hours; hbmMSCs ~1.32 and ~3.43 folds decrease; and HUCPVCs ~2.22 and ~4.58 folds decrease respectively at 24 and 48 hours post induction and compared to the controls. In-vivo imaging and PCR analysis of organs indicated that MSCs had remained at the site of injection. In conclusion, IM delivery of MSCs may be an option for the treatment of remote sites of inflammation, and would also benefit from the longer dwell time of MSC IM than is known to occur IV.

T-1003

HUMAN UMBILICAL CORD BLOOD-STEM CELLS DIRECT MACROPHAGE POLARIZATION AND LIMIT INFLAMMASOME ACTIVATION TO AMELIORATE RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is a long-lasting intractable autoimmune disorder, which has become a substantial public health problem. Despite widespread use of biologic drugs, there have been uncertainties in efficacy and long-term safety. Mesenchymal stem cells (MSCs) have been suggested as a promising alternative for the treatment of RA because of their immunomodulatory properties. However, the precise mechanisms of MSCs on RA-related immune cells are not fully elucidated. The aim of this study was to investigate the therapeutic potential of human umbilical cord blood-derived MSCs (hUCB-MSCs) as a new therapeutic strategy for patients with RA and to explore the mechanisms underlying hUCB-MSC-mediated immunomodulation. Mice with collagen-induced arthritis (CIA) were administered with hUCB-MSCs after the onset of disease, and therapeutic efficacy was assessed. Systemic delivery of hUCB-MSCs significantly ameliorated the severity of CIA to a similar extent observed in the etanercept-treated group. hUCB-MSCs exerted this therapeutic effect by regulating macrophage function. To verify the regulatory effects of hUCB-MSCs on macrophages, macrophages were co-cultured with hUCB-MSCs. The tumor necrosis factor (TNF)-α-mediated activation of cyclooxygenase-2 and TNF-stimulated gene/protein 6 in hUCB-MSCs polarized naive macrophages toward an M2 phenotype. In addition, hUCB-MSCs down-regulated the activation of nucleotide-binding domain and leucine-rich repeat pyrin 3 inflammasome via a paracrine loop of interleukin-1β signaling. These immune-balancing effects of hUCB-MSCs were reproducible in co-culture experiments using peripheral blood mononuclear cells from patients with active RA. hUCB-MSCs can simultaneously regulate multiple cytokine pathways in response to pro-inflammatory cytokines elevated in RA microenvironment, suggesting that treatment with hUCB-MSCs could be an attractive candidate for patients with treatment-refractory RA.

ADIPOSE, MUSCULOSKELETAL, AND CONNECTIVE TISSUE

T-1005

BONE AND CARTILAGE DEGENERATION IN MICE FOLLOWING LONG-DURATION SPACEFLIGHT: THE ROLE OF BONE MARROW STEM CELLS

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Exposure to mechanical unloading during spaceflight is known to have significant degenerative effects on the musculoskeletal system. Our ongoing studies with mice in short-duration microgravity experiments have identified the failure of normal stem cell-based tissue regeneration, in addition to tissue degeneration, as a significant concern for long-duration spaceflight, especially in the mesenchymal and hematopoietic tissue lineages. The 30-day US-Russian BionM1 and 37-day Rodent Research 1 (RR1) missions enabled the possibility of studying these effects in long-duration microgravity experiments. We hypothesized that the inhibition of stem cell-based tissue regeneration in short-duration spaceflight would

continue during long-duration spaceflight resulting in significant tissue alterations. High resolution MicroCT analysis of the femoral head revealed a 31% decrease in bone volume ratio, a 14% decrease in trabecular thickness, and a 20% decrease in trabecular number in the femoral head of spaceflight mice. Analysis of the regenerative potential of bone marrow osteoprogenitor cells showed increased post-flight differentiation and mineralization capacity in space-flown cells, suggesting an accumulation of osteoprecursors cells that fail to fully differentiate in space and then resume vigorous osteogenesis upon reloading. Furthermore, immunohistochemical analysis of spaceflight tissues from both BionM1 and RR1 exhibited severe disruption of the epiphyseal boundary in the femoral head, resulting in endochondral ossification and perforation of articular cartilage by bone. Microarray analysis also revealed that the top pathways altered during spaceflight include activation of matrix metalloproteinases, oxidative stress signaling and inflammation in both whole bone tissue and isolated bone marrow stem cells. In total, our results indicate that long duration mechanical unloading of bone in spaceflight results in significant, unabated, degenerative bone loss, plus the disruption of the epiphyseal boundary and endochondral ossification of the femoral head. These results suggest that the mechanical unloading of bone in microgravity may inhibit stem cell based tissue regeneration, leading to the onset of an accelerated tissue aging phenotype with signs of osteoarthritic disease.

Funding Source: Supported by NNH14ZTT001N-0062 to E. Blaber, and NNH14ZTT001N-0063 to E. Almeida.

T-1007

XENO-FREE, CLINICAL-GRADE HUMAN PLATELET LYSATE FOR MANUFACTURING HUMAN ADIPOSE-DERIVED STROMAL CELLS

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The rapidly growing interest of using human adipose-derived stromal cells (hASCs) in regenerative medicine has increased the urgency for identifying a safe, effective, and xenogeneic-free alternative to fetal bovine serum (FBS) for clinical cell manufacturing. Among the options to replace FBS, human platelet lysate (hPL) has recently emerged as a promising candidate. Compass Biomedical's PLUS™ hPL is produced under Good Manufacturing Practice (GMP) standards using expired platelet units from AABB-accredited blood banks. Growth factor levels in PLUS™ are consistent across all lots with average concentrations of 9,939±682, 641±257, 3,175±309 and 156±18 pg/mL for PDGF-BB, VEGF, EGF, and bFGF, respectively. Previous studies have demonstrated the superior effectiveness of PLUS™ hPL for manufacturing human bone-marrow derived mesenchymal stromal cells. In this study, our primary focus was to assess the capacity of PLUS™ to replace FBS for the isolation, ex vivo expansion, and cryopreservation of hASCs. To perform these experiments, fresh human lipoaspirates (n=6 donors) were obtained from Zen-Bio (Research Triangle Park, NC) and digested with 0.1% collagenase solution (Sigma, St. Louis, MO) upon receiving. The ASCs were cultured for multiple passages in three different concentrations (2.5%, 5%, and 10%) of PLUS™ and compared to those cultured in 10% FBS. We found that the hASC doubling time was approximately half as short in 5% PLUS™ as compared to 10% FBS. Based on flow cytometry analysis, hASCs cultivated in PLUS™ of all concentrations were >95% positive for stem cell markers (CD73, CD90, CD105) and ≤2% positive for hematopoietic markers (CD45, CD34, CD14, CD20), which was equivalent to FBS cultivated cells. At passage 4, PLUS™-cultivated hASCs demonstrated equivalent immunosuppressive capacity to FBS-cultivated cells as measured by a T cell proliferation assay. Cryopreserved hASCs at passage 3 using PLUS™ and dimethyl sulfoxide (DMSO) have shown comparable cell viability (>90%) and immunosuppressive capacity post-thaw when compared to FBS-cryopreserved cells. These studies demonstrate that PLUS™ hPL is a safe and reliable supplement to use in clinical applications for the isolation, expansion, and cryopreservation of hASCs without impacting their stem cell phenotype or immunosuppressive capacity.

T-1009

CULTURE OF ADIPOSE DERIVED STEM CELLS UNDER MILD HYPOTHERMIA ATTENUATES OXIDATIVE STRESS, FACILITATING EXPANSION AND DIFFERENTIATION

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Mesenchymal stromal cells (MSCs) are multipotent and can be derived from most adult tissues. Despite their many common characteristics, MSCs from different tissues display also tissue specific traits. We have recently described the tendency of visceral rat adipose derived stem cells (visASCs) to overproduce reactive oxidized species (ROS) in a NOX1 dependent manner leading to oxidative stress, apoptosis and expansion arrest. ROS overproduction was dependent on available oxygen levels since it was inhibited when cells were grown in 3% oxygen atmosphere suggesting a role for mitochondrial ROS production in visASCs oxidative stress. Reduced mitochondrial activity and ROS

production in correlation with temperature reduction was demonstrated in isolated mitochondria suggesting that temperature reduction can serve to inhibit ROS production. In addition, as was demonstrated by Dexter et al, culturing at 33oC was superior to the conventional 37oC condition in terms of longevity of bone marrow cultures and the production of hematopoietic cells. The current study was therefore aimed at investigating the effect of reduced culture temperature on ASC phenotype. We found that culturing of visASCs under mild hypothermia inhibited their ROS overproduction and prevented the consequent apoptosis and expansion arrest. Furthermore visASCs cultured under a reduced temperature demonstrated superior fat differentiation compared to 37oC visASCs. Gene chip analysis comparing 37oC and hypothermic ASCs revealed a dramatic reduction in the pro-inflammatory profile of hypothermic visASCs compared to 37oC visASCs. Thus, we show, for the first time, the effect of culture temperature on ASC phenotype and culture efficiency. We suggest that culture temperature can be used to control ASC phenotype possibly affecting both their expansion in vitro and their efficient utilization in vivo.

T-1011

IN VITRO THERAPEUTIC POTENTIAL OF FETAL BOVINE MESENCHYMAL STEM CELLS: DEVELOPMENT OF A NOVEL TREATMENT FOR BOVINE MASTITIS

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Inflammation of the mammary gland or mastitis, manly as a consequence of infectious agents including Staphylococcus aureus, is the most prevalent, costly and animal welfare threatening disease for dairy herds worldwide. Currently, treatment of mastitis is based on the use of antibiotics, aiming at controlling bacterial infection of the mammary gland. Nevertheless, this approach is not always effective and the inadequate use of antibiotics results in bacterial resistance and higher risk of antibiotic residues in milk. Thus, development of new approaches for the treatment of mastitis is imperative. The present study aimed at comparing the immunomodulatory, migratory and antibacterial properties of mesenchymal stem cells (MSC) derived from fetal bone marrow (BM-MSCs) and adipose tissue (AT-MSCs) under in vitro conditions. BM, AT and skin were surgically extracted from bovine fetuses, enzyme digested and seeded in MSC media for selection and expansion of BM-MSCs, AT-MSCs and fibroblasts (FBs), respectively. Immunomodulatory response upon interferon γ (IFNγ) stimulation was assayed for each cell line through enzymatic activity of the key immunomodulatory marker indoleamine 2,3-dioxygenase (IDO) by determination of IDO mRNA levels and N-formyl-kynurenine production. Cell migration capacity of each cell line was evaluated by scratch and transwell assays using computer-assisted image analysis (MRI Wound Healing, ImageJ NIMH, USA). The antimicrobial activity of each cell line was determined through indirect effect of MSC conditioned media (CM) on Staphylococcus aureus colony forming unit (CFU) growth after 1, 2 and 3 h of incubation. IDO mRNA and N-formyl-kynurenine levels increased ($P < 0.05$) after exposure of MSCs to IFN-γ; however, no differences were detected between MSC lines. Migratory percentages were higher ($P < 0.05$) in BM-MSCs compared to FBs; however, no differences were found between BM-MSCs and AT-MSCs. Survival percentage of Staphylococcus aureus was not affected ($P>0.05$) by CM of each cell line at any time point. However, 10X concentrated CM from BM-MSCs and AT-MSCs reduced ($P < 0.05$) survival of Staphylococcus aureus after 1, 2 and 3 h of incubation. Fetal bovine BM-MSCs and AT-MSCs displayed similar immunomodulatory, migratory and antibacterial properties under in vitro conditions.

Funding Source: National funding for the development of science and technology (Fondef grant ID15I10129), National Funding for the scientific and technological development (Fondecyt grant 1161251), Ministry of Education, Government of Chile

T-1013

CD146/MCAM DEFICIENCY DOES NOT DISRUPT SKELETAL DEVELOPMENT OR HOMEOSTASIS, BUT DOES AFFECT HEMATOPOIESIS IN MICE

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Post-natal bone marrow stromal cell (BMSC) populations contain a subset of multipotent stem/progenitor cells, able to recreate cartilage, bone, hematopoiesis-supportive stroma and marrow adipocytes, known as skeletal stem cells, SSCs. These clonogenic SSCs reside on the subluminal surface of marrow sinusoids (pericytes), and like pericytes and endothelial cells in other human connective tissues, they express CD146 (also known as melanoma cell adhesion molecule, MCAM). The role of CD146/MCAM in tumor vascularization and

invasiveness has been extensively studied, and it is currently considered to be not only a cell surface marker for cancer prognosis, but also a therapeutic target to block tumor growth and metastasis. However, the function of CD146/MCAM in pericytes in non-cancerous tissue has been relatively unexplored. Previous reports suggested that CD146 plays an important role in the association of human bone-marrow-derived pericytes (some of which are SSCs) with endothelial cells. In this study, we generated CD146-deficient mice, and focused on the bone/marrow organ. The skeletal phenotype of CD146-deficient mice was overtly normal as indicated by normal size and weight, and normal X-ray, DEXA, microCT and histological analyses. Likewise, the number of colony forming unit-fibroblasts (CFU-Fs, the closest approximation of the number of SSCs) from freshly isolated bone marrow was also normal as demonstrated by colony forming efficiency assays. Bone marrow stromal cells (BMSCs, the progeny of SSCs) were able to reform a normal bone/marrow organ upon in vivo transplantation, and bone injuries (drilled hole defects) healed normally. However, CD146-deficient mice displayed significant changes in hematopoiesis that resulted in an increase in multipotent progenitors (MPs), and a divergence of MPs into common myeloid progenitors (CMPs) and their more differentiated progeny at the expense of common lymphoid progenitors (CLPs) when subjected to hematopoietic stem and progenitor cell mobilization. Taken together, these results indicate that CD146 expression in mice is not essential for normal skeletal development, homeostasis or healing, but that its expression by SSCs/BMSCs and/or by bone marrow endothelial cells plays a role in regulating fate determination of MPs into either CLPs or CMPs. Note – Unfortunately, Prof. Bianco passed away during the course of these studies.

Funding Source: This research was supported, in part, by the NIDCR Combined Technical Core, by the DIR, NIDCR, a part of the IRP, NIH, DHHS, and by the FDA, DHHS.

T-1015

EXPERIMENTAL MODELS BASED ON HUMAN AND MOUSE ADULT STEM CELLS/LINES TO STUDY MECHANISMS OF PATHOLOGICAL REPLACEMENT OF MUSCLE TISSUE WITH ADIPOSE TISSUE

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Functions and development of skeletal muscles and adipose tissues are well coordinated and defects in this network could result in alterations in muscle regeneration and development. It is known that both tissues embryologically derive from the mesoderm; the myocyte-adipocyte cross-talks play an important role in muscle and fat development and functioning; pathological replacement of muscle tissue with adipose or fibrous tissues is observed in a number of diseases such as muscular dystrophy, age- or disease-dependent muscle degeneration. The exact molecular mechanisms of replacement of muscle with fat are not known. In our work we aimed to develop experimental model to study in vitro the "switch" between stimulation of muscle regeneration and muscle replacement with adipose tissue. Different skeletal muscle progenitor cells were used in this study: mouse satellite cells (mSC), muscle stromal cells (known as FAP), mouse myoblasts C2C12; human satellite cells (hSC) and human muscle stromal cells (hStMC) from healthy donors. Human intramuscular adipose stromal cells (hIMAdSC) and hStMC from DMD patient (DMD-hStMC) served as a fat differentiation and impaired muscle regeneration models respectively. All types of cells were evaluated for differentiation potential. Myogenesis: in all muscle progenitors including DMD-hStMC serum starving resulted in sequential stimulation of expression of early (Mif5, MyoD) and late (MyoG, Mrf4) regulators of myogenesis. Formation of myotubules positive for MHC immunostaining detected by day 3-4. Adipogenesis stimulated with modified adipogenic cocktail (MAC): FAP and hIMAdSC differentiated into adipocytes under MAC treatment; that confirmed by OilRed staining and expression of markers and regulators of adipogenesis (C/EBPa, PPARg, Fabp4, PLIN1). In mSC, hSC, hStMC, and C2C12 MAC stimulated differentiation not adipocytes but myotubules positive for MHC and desmin immunostaining. In DMD-hStMC MAC didn't induce myogenesis. We have demonstrated here that the same stimuli could induce both myogenesis and adipogenesis depending on cell type and culture context and coordinate the balance between adipocytes and myocytes in complex tissue like skeletal muscle.

Therefore, this system is useful to study the mechanisms of pathological replacement of muscle tissue with fat.

Funding Source: Work was funded by Russian Science Foundation under agreement 16-15-10178

T-1017

LOSS OF TARGET MUSCLE STEM CELLS DRIVES AGE-RELATED NEUROMUSCULAR SYNAPSE DETERIORATION IN MICE

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A prominent aspect of sarcopenia, the age-associated loss of skeletal muscle integrity and function, is the deterioration of neuromuscular synapses, specialized sites of motor neuron innervation. It is well established that age-related neuromuscular synapse remodeling is connected to skeletal muscle maintenance and function. Although this decline has been attributed to denervation, whether alterations in target muscle cells, and/or motor nerves primarily drive this process is unknown. Initially we examined gene expression profiles and neuromuscular synapse integrity in aged skeletal muscles. Despite displaying a high level of neuromuscular synapse remodeling normally ascribed to cycles of denervation/reinnervation, aged skeletal muscles did not possess a gene expression signature coherent with denervation/reinnervation. Instead, degenerated neuromuscular synapses were associated with reductions in the size of post-synaptic myonuclear clusters, consistent with target muscle fiber degeneration. Since the regenerative potential of skeletal muscle is endowed in a population of Pax7-expressing resident stem cells, satellite cells, we utilized fate tracking, depletion, and rescue strategies to determine whether age-related neuromuscular synapse deterioration is connected to loss of satellite cells. Fate-tracking strategies conducted within a relatively short time revealed that satellite cells contribute to neuromuscular synapses in young adult and middle-aged mice, and these contributions were specifically lost at more advanced ages. In addition, we demonstrated that satellite cell contributions are vital to lifelong maintenance of neuromuscular synapses, as satellite depletion was sufficient to induce neuromuscular synapse degeneration and associated muscle fiber atrophy at a younger age. Finally, prevention of satellite cell loss was able to attenuate aged neuromuscular synapse degeneration and promote aged muscle force generation. Our observations demonstrate that alterations in target muscle cells provide the cellular basis for age-related neuromuscular synapse remodeling and associated skeletal muscle decline.

Funding Source: This work was supported by URMC start-up funds and NIH grant (RO1AG051456).

T-1019

FUNCTIONAL SCREENING OF COMMON GENETIC VARIANTS ASSOCIATED WITH INSULIN RESISTANCE INDEPENDENT OF OBESITY IN HSGBS PREADIPOCYTES

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Insulin resistance is one of the most common risk factors for type 2 diabetes (T2D). Due to the strong correlation between obesity and insulin resistance, the relative role of insulin resistance in T2D, independent of obesity, has been more difficult to disentangle. However, in a genome-wide association study of up to 133,010 individuals of European ancestry without diabetes, common genetic variants were discovered to be associated with insulin resistance with eQTLs in adipose tissue independent of body size. Functional validation of these susceptible candidate genes would improve our understanding of the contributions of insulin resistance to T2D in both obese and non-obese individuals. In this study, we have established a high-throughput functional screening platform using CRISPR/Cas9 technology. Briefly, 16 candidate genes were targeted in human Simpson-Golabi-Behmel Syndromes (HSGBS) preadipocyte. After 20 days of differentiation, adipocytes were used to test their insulin response. Preliminary data showed that the 16 isogenic HSGBS knockout lines had different proliferation capacity and differentiation efficiency, while the corresponding adipocytes indicated distinct levels of triglycerides, free fatty acid, glucose uptake and insulin signaling in response to insulin stimulation, suggesting different levels of insulin sensitivity.

T-1021

ROLE OF MICRO RNA-302 FAMILY IN MESENCHYMAL STEM CELL DIFFERENTIATION TO OSTEOGENIC AND ADIPOGENIC LINEAGES

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The aims of this study were to investigate the role of micro RNAs (miRNAs) in the differentiation of mesenchymal stem cells (MSCs). Human bone-

marrow derived MSCs were cultured and treated with osteogenic or adipogenic media and miRNA expression tested using miRNA array. We identified 20 miRNAs that were differentially regulated during differentiation, 8 of which had not previously been reported to be involved in MSC differentiation. From these we selected 2 family members of miRNA-302 (a and b) for further investigation. Firstly we validated these results by qRT-PCR and found that miRNA-302a and b were down regulated during differentiation. To determine the role of the miR-302 family during MSC differentiation, their functional activity was tested by knockdown and over expression, via transfection of miRNA-302 family inhibitor or mimic into MSCs and the cells were treated with appropriate media for 14 days. Then lineage specific gene expression was measured by qRT-PCR. Knock-down of miRNA-302 family in MSCs caused a significant upregulation of between 2.5-3.5 times of RUNX2 expression and between 3-8.5 times of ALP expression during osteogenesis of MSCs. However, there were no notable effects of miR-302 knockdown after treating MSCs with adipogenic medium. On the other hand, overexpression of miR-302b did not make any significant changes in osteogenic specific gene expression during differentiation. The results suggest that the miR-302 family may act to maintain MSC naivety and regulate osteogenic gene expression. However, miR-302b may not directly affect MSC differentiation into osteoblasts and suggests intra-familial miRNA differences may be present that have distinct effects upon cell fate.

T-1023

POLYSOME PROFILING REVEALS CONTINUOUS TRANSLATION OF ACTIVATION FACTORS IN QUIESCENT MUSCLE STEM CELLS

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Under homeostatic conditions, muscle stem cells remain quiescent in their niche, but the molecular mechanisms by which these cells maintain the quiescent state are not well understood. Recent studies have used transcriptomics as a proxy for protein levels in cells to investigate the quiescent state. However, due to variable modes of regulating mRNA translation and stability, the correlations between transcript and protein levels are often low. Here we present the first global analysis of the quiescent translatome. To determine which transcripts are translated by quiescent muscle stem cells in vivo, we analyzed the polysome-bound transcripts using the RiboTag approach. Among the transcripts associated with actively translating polysomes, we identified known quiescence markers as well as regulators of mRNA stability and proteostasis. Surprisingly, we also detected the presence of transcripts encoding differentiation factors such as MyoD1 for which the actual protein is undetectable. We show that a majority of muscle stem cells translate the MyoD protein in the quiescent state in vivo, only to break it down via ubiquitination and proteasomal degradation. Moreover, our data suggest that muscle stem cells use translation-degradation coupling as a global mechanism to limit protein levels of differentiation factors, possibly to maintain a quiescent state. Our data show that, in quiescent muscle stem cells, transcript level is only weakly linked to translation and that there are post-translational mechanisms in place to regulate the levels of activation-related proteins.

CARDIAC TISSUE

T-1025

FILLING THE GAP FOR TRANSLATION OF IN VITRO ASSESSMENTS TO CLINICAL OBSERVATIONS OF DRUG-INDUCED CARDIAC ARRHYTHMIAS AND LONG TERM TOXICITY

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Human induced pluripotent stem cell (iPSC)-derived cardiomyocytes represent a promising model for in vitro prediction of cardiac arrhythmias. Currently, commercially available hiPSC-derived cardioymocyte products are being validated in an international multi-coresite study inside the Comprehensive in vitro Pro-arrhythmia Assay (CiPA) consortium. This aims to change the paradigm of safety pharmacological assessment from that of focus on the hERG channel and the potential to delay repolarization now to a potentially clinically more relevant assay system using human iPSC-derived cardiomyocytes in MEA-, voltage- and calcium-sensitive dye recordings. Besides CiPA, a variety of oncological drugs including tyrosine kinase and HDAC inhibitors have been reported to induce long-term cardiac toxicity in the clinic. Recent findings clearly show that iPS-derived cardioymocytes employed in a long-term impedance assay provide an almost perfect recapitulation of the observations of clinical drug-induced cardiotoxicity. Here we show the cardiac toxicities resulting from iPS cardiomyocytes being treated with 5 different HDAC inhibitors under sub-acute dosing conditions. The results shown here imply that human iPSC-derived Cor.4U human cardioymocytes are a widely employed translational in vitro cell model

for the prediction of clinically relevant drug-induced cardiac arrhythmias and long-term toxicities.

T-1027

MAJOR HISTOCOMPATIBILITY COMPLEX-I DEFICIENT EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES AS POTENTIAL OFF-THE-SHELF THERAPY FOR CARDIAC REGENERATION

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Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) can regenerate myocardium as they can engraft, electrically couple and remuscularize. Optimally, hESC-CMs would be banked as a source of immediate off-the-shelf therapy. However, hESC-CMs have intrinsic immunoreactivity, which would require immunosuppressive regimens to prevent rejection. A significant cause of graft rejection is the recognition of the major histocompatibility complex class I (MHC-I). Cells devoid of cell surface MHC-I through deletion of beta-2 microglobulin (B2M, sine qua non required molecule for MHC-I surface expression) may be able to avoid an immune response. We therefore decided to test the differentiation potential and immunogenicity of B2M-/- hESC's; and also, to test an hESC-based cardiac regenerative therapy by utilizing CMs derived from MHC-I deficient murine neonatal cardiomyocytes (NCM). Undifferentiated B2M+/+ and B2M-/- hESCs were differentiated into cardiomyocytes and tested for cell mediated immunogenicity and expression of cardiac troponin (cTn) and MHC-I. B2M+/+ and B2M-/- pregnant females were given BrdU to label NCM. B2M+/+ and B2M-/- NCMs were injected into the left ventricles of adult mice. B2M+/+ and B2M-/- hESCs were differentiated into CM's using small molecule-based protocols resulting in 80-90% positivity for cTn-I, cTn-T, α-myosin heavy chain and NKX2.5 cardiac transcription factor. MHC-I expression was low in CMs at baseline; however, in B2M+/+ CM's there was a robust increase in MHC-I expression in response to interferon-γ and tumor necrosis factor-α, which was not seen in B2M-/- CM's. Consistently, B2M-/- CMs did not trigger CD8+ or CD56+ NK-cell response in vitro. There was no evidence of rejection in hearts transplanted with B2M-/-NCM seen up to 5 weeks post injection. In contrast, hearts transplanted with B2M+/+ NCM's showed signs of myocardial death at 3-7 days. BrdU positive cells were seen in areas of necrosis. hESCs-derived CM derived from B2M-/- showed no expression of MCH-I and had a markedly attenuated immune response in vitro. This reduced immunity was also seen with B2M-/- NCMs which evaded rejection in vivo. This study suggests that B2M-/- hESCs may be a good strategy for the development of universal cell therapies for cardiac regeneration.

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T-1031

SWITCH FROM FETAL TO ADULT SCN5A ISOFORM IN HIPSC-DERIVED CARDIOMYOCYTES UNMASKS THE CELLULAR PHENOTYPE OF A CONDUCTION DISEASE-CAUSING MUTATION

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While human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) can recapitulate features of some ion channel mutations causing inherited rhythm disease, the electrophysiological immaturity of these cells still represents a significant limitation of the model. Prolongation of the time in culture is known to increase the acquisition of more mature characteristics. SCN5A, the gene encoding the alpha subunit of the Na-channel, undergoes a developmental maturation by switching from the "fetal" to the "adult" transcript isoform, which differ only in the exon 6 sequence. A mutation I230T, localized only in the adult exon 6, is carried by some individuals of a family with recessive cardiac conduction

disease. We investigated the relationship between the expression fraction of 'adult' SCN5A isoform and the electrophysiological phenotype of this mutation at different time points during an extended culture period in hiPSC-CMs generated from a homozygous (I230Thomo) and a heterozygous (I230Thet) carrier. After 20 days of culture, the Na+-current (INa) was mildly reduced in I230Thomo-hiPSC-CMs compared to control hiPSC-CMs, while I230Thet- hiPSC-CMs displayed no reduction in INa. This coincided with a relatively high expression fraction of the 'fetal' SCN5A isoform compared to the 'adult' isoform as measured by qPCR. Upon 66 days of culture, the fraction of 'adult' SCN5A isoform increased; this was paralleled by a marked decrease in INa in I230ThomohiPSC-CMs. At this time in culture, I230Thet hiPSC-CMs displayed an intermediate loss of INa, compatible with a gene dosage effect. In conclusion, we show an increased expression fraction of the 'adult' sodium channel isoform upon prolonged culture of hiPSC-CM. Therefore the electrophysiological phenotype of a mutation in an ion channel can be enhanced and unmasked by the acquisition of a more mature cellular state over time.

T-1033

THE DEVELOPMENTAL ORIGIN OF CARDIAC FIBROBLASTS INFLUENCES THE EFFICIENCY OF DIRECT REPROGRAMMING TO CARDIOMYOCYTES

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Direct conversion of fibroblasts to cardiomyocytes (CM) is advancing the field of cardiac regeneration. Despite all the advantages of direct reprogramming, the presence of residual epigenetic memory of the original cell population may be a hurdle in bringing this technology closer to the clinic. Thus, choosing a starting cell with a similar ontogeny to the desired reprogrammed cell may overcome some of the limitations. Expression of key cardiogenic genes shared between cardiac fibroblasts (CFb) and CM in addition to the abundance of these cells, suggest that CFb may be the optimal autologous cell source for therapeutic manipulation in treatment of heart disease. We hypothesized that progenitors, transiently expressing Mesp1 generate a sub-population of CFb which are more prone to direct reprogramming and adopt a cardiomyocyte gene profile due to their maintained epigenetic memory. We generated a Mesp1cre;R26mTmG mouse to label all cells expressing Mesp1 and their progeny and we observed that the majority of the cells in the heart including CFb are derived from Mesp1 cells. We showed that ~80% of resident CFb are derived from Mesp1 while a minor non-Mesp1 subset also exists. We compared the reprogramming efficiency of CFb of Mesp1 origin to CFb of other origin to induced CM (iCM) by overexpression of specific cardiac transcription factors. Results from immunostaining and gene analysis showed higher expression of cardiac muscle markers in CM induced from Mesp1 CFb. To further delineate potential differences between two subsets, we performed RNAseq and our results showed that the non Mesp1 CFb were enriched in neural crest related genes. We generated Pax3Cre;R26mTmG mice to lineage trace neural crest-derived cells. Our results confirmed a minor contribution of Pax3 cells to CFb. We developed a modified CLARITY technique to transform the heart into an optically-transparent organ for light-sheet fluorescence imaging. We observed that Pax3 CFb were mainly located in the wall of aorta while Mesp1 CFb were distributed throughout the heart. Additionally, we are studying whether each CFb subset has the tendency to generate a specific subtype of iCM (ventricular, atrial, and nodal CM). These results can identify distinct sub-populations of CFb, which can generate functional cardiomyocytes for cardiac-regenerative therapies.

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T-1035

STANDARDIZED GENERATION, PURIFICATION AND QUALITY CONTROL OF HUMAN PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

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Fast and highly efficient generation of pure pluripotent stem cell derived cardiomyocytes (PSC-CMs) is a prerequisite for clinical applications, drug development and several research applications, e.g. heart disease modeling. In recent years, various protocols to differentiate PSCs into cardiovascular cells such as cardiomyocytes (CMs) have been published. However, cardiovascular differentiations of human PSC cultures do not contain homogeneous cell populations, but are rather composed of a variety of CMs and non-CMs including different CM subtypes or subpopulations. The cell composition of each differentiation is currently depending on the stem cell clone, its passage, differentiation protocol used and additional experimental parameters. To circumvent these experimental variations and prepare for standardized processes suitable for automation and clinical scale up, we have established a new workflow ranging from controlled cardiac differentiation to CM harvesting, purification, analysis, replating and freezing. This work included the development of a robust, fast and highly

reproducible differentiation protocol, yielding in cardiac differentiation efficiencies of 70% within less than 10 days of differentiation. To further purify PSC-derived CMs, we have developed a gentle harvesting method for PSC-derived cardiomyocyte monolayers and established both magnetic and flow cytometry-based purification protocols delivering PSC-CMs in purities of up to 97%. For quality control of differentiation and separation processes, we have engineered antibodies allowing for fast and easy characterization of PSC derived cardiomyocytes and their subpopulations in immunofluorescence and flow cytometry applications. Additionally, a novel cryopreservation method for PSC derived CMs allows for standardized freezing and thawing of PSC-CMs with high viabilities. Taken together, the newly established workflow solves several technical issues related to the generation of PSC-derived cardiomyocytes. In the next step, the process will be transferred into a scalable, automated closed system.

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

T-1037

FUNCTIONAL CHARACTERIZATION OF HUMAN PLURIPOTENT STEM CELL -DERIVED ARTERIAL ENDOTHELIAL CELLS

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Here, we report the derivation of arterial endothelial cells from human pluripotent stem cells that exhibit arterial-specific functions in vitro and in vivo. We combine single-cell RNA-sequencing of embryonic mouse endothelial cells with an EFNB2-tdTomato/EPHB4-EGFP dual reporter human embryonic stem cell line to identify factors that regulate arterial endothelial cell specification. The resulting xeno-free protocol produces cells with gene expression profiles, oxygen consumption rates, nitric oxide production levels, shear stress responses, and TNFα-induced leukocyte adhesion rates that are characteristic of arterial endothelial cells. These arterial endothelial cells were found to significantly improve animal survival in a myocardial infarction model. Arterial endothelial cells were robustly generated from multiple human embryonic and induced pluripotent stem cell lines and have potential applications for both disease modeling and regenerative medicine.

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T-1039

THE ENDURING MYSTERY OF THE 9P21 LOCUS: IMPLICATION OF THE LONG NON-CODING RNA ANRIL IN CORONARY ARTERY DISEASE PREDISPOSITION

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In 2007, three independent GWAS studies identified a locus on human chromosome 9p21 which DNA variants are the most strongly associated with Coronary artery disease. Interestingly, these DNA variants are not associated with traditional risk factors for myocardial infraction, suggesting the existence of a novel mechanism that could represent a new therapeutic target. Furthermore, these variants are associated with both aortic and intracranial aneurysm, suggesting a shared connection between the locus and the vascular pathology of the disease. The disease-associated single nucleotide polymorphisms (SNPs) are all located in a gene poor region comprising only a long non-coding RNA (lcnRNA) called ANRIL, raising the idea that risk variants modulate CAD predisposition by regulating ANRIL activity. To test this hypothesis we generated homozygous null human induced pluripotent stem cell (hIPSCs) lines using genome editing (CRISRP/Cas9) and differentiated them into endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). As increasing data suggest that lncRNA can regulate the transcription of nearby genes in cis or affect gene expression in trans, we performed a gene expression comparison (RNA-seq) of ANRIL knockout hPSC-ECs/VSMCs and their isogenic wild-type counterparts. Preliminary data suggest that ANRIL plays a role in VSMCs proliferation, contractility and adhesion. We are currently using functional assays to confirm these RNA-seq data. Finding by which mechanism(s) risk variants within the 9p21 locus confer CAD susceptibility could lead to new diagnostic tools and therapeutics.

HEMATOPOIESIS/IMMUNOLOGY

T-1041

HIGH LEVEL HGF CONTRIBUTES TO BOTH IN VIVO AND IN VITRO HEMATOPOIETIC SUPPORTIVE ACTIVITIES OF HUMAN FETAL LIVER-DRIVED MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSC) derived from various tissues have shown different ability to support and maintain hematopoiesis in vivo and in vitro. However, mechanisms in support of this activity are not fully characterized. In the present study, the hematopoietic supportive activities of MSC derived from second trimester fetal liver and bone marrow were characterized and the underlining mechanism was studied. in vivo hematopoietic activity was assessed with NOD/SCID mouse transplanted with human UCB-CD34+HSPC and co-transplanted with BM-MSC or FL-MSC; in vitro hematopoietic supporting activity was assessed with long term co-culture with human UCB-CD34+HSPC. FACS assay of human CD45+ cells from BM, PB and spleen of the transplanted NOD/SCID mouse suggested that MSCs from both fetal tissues support hematopoietic reconstitution. Intriguingly, the fetal liver MSC demonstrated much stronger supportive activity than that of bone marrow MSC (> 2 fold). To explore the molecular mechanism, comparison of gene transcriptome and secreting cytokine have been carried out. Among CCL11、RANTES、MCP-1/3 and GRO, HGF is the cytokine that most highly expressed in FL-MSC and was further investigated its activities. when human UCB-CD34+HSPC co-transplanted with HGF-shRNA expression lenvirus transfected FL-MSC into NOD/SCID mice for 4-6 weeks, CD45+ and CD34+ human HSPC are reduced to 60% and 40% of control, respectively. Our data suggest that HGF is important for the fetal liver hematpoietic development and fetal liver MSC is an important niche component.

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T-1043

A SHORT PULSE OF PROSTAGLANDIN E2 (PGE2) INDUCES LONG TERM SELF-RENEWAL BY PREVENTING EXHAUSTION OF HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cells (HSCs) are the most widely used stem cell treatment, yet their numbers are often limited. Prostaglandin E2 (PGE2) has been previously identified as a potent enhancer of functional HSCs, a treatment that is showing promise in clinical trials. We further elucidated PGE2’s mechanism in a mouse transplantation setting. The HSC compartment (Lineage-, Sca1+, cKit+, CD150+, CD48-) consists of a CD34 high (=CD34HI) and CD34 low (=CD34LO) population. Competitive transplantation assays confirmed that increasing CD34 expression leads to diminishing long-term engraftment. By RNAseq CD34HI HSCs express higher levels of cell cycle genes compared to the CD34LO population likely resulting in exhaustion of transplanted CD34HI cells. When treated with PGE2 for 2 hours ex-vivo CD34HI HSCs had both increased chimerism and prolonged engraftment upon competitive transplantation. The long term effect persisted in competitive secondary transplants. Next, mice were subjected to a 5-FU challenge followed by PGE2 ex-vivo treatment and competitive transplantation of whole bone marrow. Chimerism of 5-FU donor marrow that had received an additional 2h PGE2 pulse was significantly increased compared to 5-FU only control. To investigate the underlying molecular mechanism phospho-FACS indicated that CREB is phosphorylated immediately downstream of PGE2 within 15 min of treatment. Using Chip-seq in human CD34+ cells, PGE2 induced CREB binding could be correlated with chromatin remodeling such as changes in the histone mark H2K27ac. Using ATAC-seq we determined novel open chromatin sites that were exposed in response to PGE2 treatment. We found an enrichment of CREB and AP-1 motifs both in PGE2 treated human CD34+ cells and mouse CD34HI HSCs. RNAseq data indicated differential expression of a number of AP-1 genes such as Fosl2, Fos, JunB and Jun both immediately after PGE2 stimulus as well as in treated and transplanted CD34HI HSCs. Our model suggests that PGE2 acts through CREB to induce acute chromatin changes (e.g. exposing AP-1 sites) that leads

to a permanent fate change prolonging CD34HI HSC engraftment by preventing exhaustion. Unravelling PGE2's effect on HSCs will further current understanding of basic HSC biology and add to clinical applications targeted at improving hematopoietic transplants.

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T-1045

ACTIVELY CYCLING MURINE LINEAGE POSITIVE BONE MARROW CELLS CONTAIN LONG-TERM MULTI-LINEAGE ENGRAFTMENT CAPACITY

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We have found a population of actively cycling hematopoietic stem cells (HSCs) in murine whole bone marrow (WBM) capable of long-term multi-lineage engraftment. Our data indicate that these cells are lost with conventional stem cell purification and are recovered, in part, within the total lineage positive (Lin+) fraction. The goal of these studies was to further characterize this understudied population of cycling HSCs within the Lin+ population. We first evaluated the engraftment capacity within distinct Lin+ subsets. Although conventionally felt to be devoid of significant stem cell capacity, we found that populations of myeloid (GR1 or CD11b+), erythroid (TER119+) and B-lymphoid (B220+) cells isolated from B6.SJL (CD45.1) donor mice, when competitively engrafted into lethally irradiated C57BL/6 (CD45.2) mice, all contained multi-lineage engraftment capacity 6 months post-transplant. The average % donor chimerism ± SD in the myeloid, erythroid, and B-lymphoid groups was 14%±16%, 27%±21% and 13%±15%, respectively (1-2x106 donor + 3x105 C57BL/6 WBM cells). To evaluate more purified Lin+ subsets, we double sorted the B220+, CD11b+, and GR1+ cells and collected the following two populations: 1) those cells remaining positive for the Lin+ marker on double sort and 2) those cells now negative for the single Lin+ marker. At 6 months post-transplant, we found a reduction in average %donor chimerism in the persistently positive Lin+ subsets on double sort (0.1%-1.6%). However, there was significant engraftment potential within those cells that were negative for only the single Lin+ marker on double sort (31%-59% average % donor chimerism). Using tritiated thymidine suicide to assess cell cycle status, we found that the engraftment capacity within the population of cells that were B220+ on primary sort and became negative on double sort was entirely due to cycling stem cells. These data indicate that the stem cell capacity within this population cannot be due to highly purified quiescent HSCs contaminating our primary Lin+ sort. As primary sorted Lin+ cells comprise up to 98% of WBM, we think engraftment capacity within the primary sorted Lin+ fraction discarded with conventional HSC purification represents a potentially large and understudied stem cell population within WBM worthy of further characterization.

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T-1047

TRANSITION OUT OF HSC DORMANCY BY A CONTINUOUS UPREGULATION OF METABOLISM IS CONTROLLED VIA RETINOIC ACID

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Hematopoietic stem cells (HSCs) harbor the capacity to generate a series of multipotent progenitors (MPPs) that differentiate into lineage-committed progenitors and subsequently mature cells. To explore essential HSC features, we recently integrated quantitative proteome, transcriptome, and methylome analyses of five FACS-sorted HSCs and MPP populations (MPP1-4) and combined these OMICs analyses to their functional potential (Cabezas-Wallscheid et al., Cell Stem Cell 2014; Klimmeck et al., Stem Cell Reports 2014; Lipka et al., Cell Cycle 2014). We have now expanded this analysis to dormant HSCs (dHSCs) identified by label-retaining assays (Wilson et al., 2008). Rare dHSCs reside at the top of the blood hierarchy harboring the highest long-term reconstitution capacity. However, till the date the molecular identity of dHSCs, as well as the mechanism regulating maintenance and the transition out of dormancy remain unknown. We now show by single-cell RNA-seq analysis that the transition from dormancy towards cell cycle entry is achieved by a

continuous and coordinated up-regulation of all major biosynthetic processes rather than a switch on/off mechanism. We generate a novel transgenic reporter mouse that specifically labels dHSCs avoiding label retention assays and we demonstrate by in vitro and in vivo approaches the relevance of retinoic acid signaling to keep HSC dormancy.

T-1049

IDENTIFYING AND ENRICHING THE PLATELET-PRODUCING HUMAN STEM CELL-DERIVED MEGAKARYOCYTES USING FACTOR V UPTAKE

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Stem cell-derived platelets have the potential to replace donor platelets for transfusion. Defining the platelet-producing megakaryocytes (MK) within the heterogeneous MK culture is important for optimizing in vitro platelet generation. Using two human stem cell models (CD34+ hematopoietic stem cells and induced pluripotent stem cells (iPSCs)), we identified novel MK populations corresponding to distinct maturation stages. An immature, low granular (LG) MK pool (defined by side scatter on flow cytometry) gives rise to a mature high granular (HG) pool, which then becomes damaged by apoptosis and GPIbα (CD42b) shedding. The undamaged HG/CD42b+ MK subpopulation has features consistent with mature MKs, including high ploidy, high RNA, alpha-granular content and maximum responsiveness to agonist stimulation. The mature HG/CD42b+ MKs endocytose fluorescently-labeled coagulation factor V (FV) from the media into alpha-granules and release functional FV+CD42b+ human platelet-like particles, when infused into immunodeficient mice. Importantly, these FV+ particles have the same size distribution as infused human donor platelets and are preferentially incorporated into clots after laser injury. Using drugs to protect HG MKs from apoptosis and CD42b shedding, we also demonstrate that apoptosis precedes CD42b shedding and that blocking apoptosis enhances platelet yield. These studies identify a transition between distinct MK populations in vitro, including one that is primed for platelet release. Technologies to optimize and select these platelet ready MKs may be important to efficiently generate functional platelets from in vitro-grown MKs.

T-1051

MEGAKARYOCYTE V.S. ERYTHROID FATE SPECIFICATION BY CELL CYCLE REGULATION

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In healthy adults, there are about 25 x 10^12 RBCs and 1.5 x 10^12 platelets circulating, which result from lineage specific differentiation of Megakaryocyte-Erythroid Progenitors (MEP). Control of RBC and platelet numbers is highly regulated. Although several transcription factors and cytokine signaling pathways have been shown to affect differentiation of cells down the megakaryocytic (Mk) and erythroid (E) lineages, detailed molecular mechanisms underlying the MEP fate decision have not been determined. In our previous report, we develop a unique high purity isolation strategy for MEP, megakaryocyte progenitors (MkP) and erythroid progenitors (ErP) from either human bone marrow or G-CSF mobilized peripheral blood cells. We performed single-cell deep sequencing on these cell populations and found that cell cycle genes are significantly differentially regulated between MEP, MkP, and ErP. When we sorted primary human cells based on CFSE dilution, we found significant differences in cell cycle behavior of MEP that become E committed vs. Mk committed. To confirm that cell cycle regulation might affect the bi-potent MEP fate decision, we interfered with cell cycle speed by overexpression of cyclins or knock down of MAX, a heterodimer partner of the oncogene MYC. Similarly, several small molecules that slow the cell cycle by different mechanisms were used to assess effects on the MEP cell fate decision in vitro. The results indicate that the MEP fate decision was significantly altered when the cell proliferation rate was altered. Consistent with these data, we show that as primary mouse MEP commit to the Mk vs. E lineage in vivo, there are significant differences in their cell proliferation rates. Over all, our results indicate that cell cycle regulation play a significant role in the MEP fate decision mechanism. Elucidation of the mechanism of MEP fate decision events in closely related progenitor populations in from healthy subjects increases our knowledge of what may occur in many benign and malignant disease states of Mk or E production such as Essential Thrombocytosis and leukemia.

Funding Source: Yale Cooperative Center of Excellence in Hematology Pilot & Feasibility award

T-1053

DEFINING THE ROLE OF ARYL HYDROCARBON RECEPTOR SIGNALING IN HEMATOPOIESIS USING A CHEMICALLY-DEFINED HUMAN PLURIPOTENT STEM CELL MODEL PRIMED FOR DEFINITIVE HEMATOPOIESIS

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A longstanding goal within the hematopoietic stem cell (HSC) field is to fully define the conditions required for the efficient modeling of definitive hematopoietic development in vitro utilising human pluripotent stem cells (hPSC). We have previously reported the highly efficient generation of primitive/fetal-type erythrocytes from human induced pluripotent stem cells (hiPSC) via stage-specific activation of the aryl hydrocarbon receptor (AHR) pathway. Here we describe a robust, chemically-defined 2-D differentiation protocol for the generation of hematopoietic progenitor cells (HPCs) from hiPSC. The HPCs are enriched for CD34+CD45+ cells that demonstrate myeloid, erythroid and lymphoid potential in a series of assays. Critically, the erythrocytes generated from hiPS-HPCs express several magnitudes higher levels of HBB (adult, β-globin) and concomitantly, downregulation of HBG2 (foetal, γ-globin) and HBE (embryonic ε-globin) compared to the hiPS-erythrocytes we previously characterized. In agreement with what is known about the role of notch signaling in the emergence of definitive hematopoiesis, stage-specific small-molecule inhibition of notch signaling leads to a dramatic downregulation in β-globin expression in the resultant erythroid cell population. We are currently utilizing this platform to study the role of AHR signaling in developmental primitive and definitive hematopoiesis. AHR signaling in the HSC compartment is of interest within the field as several studies report that small molecule inhibition of AHR signaling leads to the expansion of functional CD34+ stem/progenitor cells in in vitro cultures. To more closely examine the role of AHR signaling in hematopoietic development, we have generated AHR -/- hiPSC lines via CRISPR-Cas9 gene editing. AHR -/- hiPSC generate increased numbers of hematopoietic cells compared to WT hiPSC, and increased proportions of these cells are CD34+CD45+. Interestingly, we find that significantly fewer AHR -/- HPCs express KIT compared to WT-hiPSC HPCs. These findings mirror our observations with AHR inhibitor compounds in this system. We are currently mapping the expression of the AHR throughout human hematopoietic development while defining the mechanism by which it impacts development and specification.

T-1055

DIRECTING B AND T CELL DIFFERENTIATION FROM INDUCED PLURIPOTENT STEM CELL-DERIVED HEMOGENIC ENDOTHELIUM

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Autologous cell therapy for the treatment of hematological malignancies and primary immunodeficiencies has been limited by the inability to yield functional human hematopoietic stem cells (HSCs) from induced pluripotent stem cells (iPSCs) in vitro. To explore factors that confer functional HSCs, we induced hemogenic endothelium (HE), the fetal precursors to HSCs, from iPSCs. Here, we identify five transcription factors (RUNX1, ERG, LCOR, HOXA5, HOXA9) that enable further conversion to HSC-like cells with lymphoid potential in vitro and multilineage engraftment capacity in vivo. We conducted a candidate screen of small molecules aimed at enhancing HSC conversion in a RUNX1c gene reporter assay. In the candidate screen, IFN-γ enhanced RUNX1c reporter 2.4-fold, which was confirmed by flow cytometry showing increased hematopoietic progenitor cells (CD34+CD45+). These findings suggest that transient exposure to IFN-γ may bolster the typically low engraftment efficiency of iPSC-derived HSCs. Our approach to creating engraftable HSCs holds significant potential for modeling hematopoietic disease, both in animal models and as a "disease-on-a-dish," and for developing therapeutic strategies in genetic blood disorders.

Funding Source: Howard Hughes Medical Institute; American Society of Hematology

T-1057

INVESTIGATING HUMAN HEMATOPOIESIS USING HUMAN EMBRYONIC STEM CELLS

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One of the major challenges in the field of regenerative medicine and stem cell therapy research is the expansion of Hematopoietic Stem Cells (HSCs) ex vivo and their de novo generation in vitro. HSCs are multi-lineage self-renewing progenitors, which provide a life-long supply of all mature blood cells. Thus, transplantation of in vitro-derived HSCs would greatly benefit patients affected by hematological diseases and other types of cancer. Our group has been optimizing cell culture conditions to promote the differentiation of human Embryonic Stem Cells (hESCs) into hematopoietic progenitor cells from hemogenic endothelial population, through an

endothelial-to-hematopoietic transition. This system closely mimics in vivo blood cell emergence and offers a great model to dissect embryonic hematopoiesis and a potential source of hematopoietic stem and progenitor cells for therapeutic purposes. Our current interest is to optimise this in vitro differentiation system and identify crucial factors involved in hematopoietic development. In particular, we are currently investigating the heterogeneity of hESC-derived CD31+ CD144+ progenitor cells, as they retain remarkable hematopoietic potential. We hypothesize that this population consists of a heterogeneous pool of endothelial progenitors with various differentiation potential and our aim is to better characterize the hemogenic endothelium subset within this population. To this end, we are analysing the biological potential of the population of interest using limiting dilution assays and single-cell cultures. Additionally, we are applying the recent advances in the field of single-cell RNA sequencing to assess the level of heterogeneity at the transcriptome level. Our results will shed light on the dynamics of in vitro-generated hematopoiesis and will lead to the development of more efficient protocols for the derivation of bona fide HSCs.

T-1059

SIMVASTATIN-CONJUGATED NANOPARTICLE ENHANCES THE THERAPEUTIC EFFECT OF ADIPOSE-DERIVED STEM CELLS ON INTERSTITIAL LUNG DISEASE

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Clinical trials of autologous adipose-derived stem cell (AdSC) therapy for interstitial lung disease (ILD) are now on-going. We have investigated the hypothesis that statin, an agent with pleiotropic effects, could augment the therapeutic potential of AdSCs.Simvastatin-conjugated nanoparticles (STNP) significantly promoted the migration activity and cell survival without changing the proliferation activity, and up-regulated Transforming Growth Factor (TGF) -β1 in vitro assays. Next, ILD was induced by bleomycin (BLM) in C57BL/6 mouse, and the mice were assigned in the following groups: 1) Control, 2) NP-AdSCs (25000 cells), and 3) STNP-AdSCs (25000 cells). Lung inflammation and fibrosis assessed were significantly supressed at 4 weeks after starting BLM administration in STNP-AdSC group. The levels of IL-4, IFN-gamma, TNF-alpha, COL1A1, and TIMP1 mRNA expression at 28 days after BLM administration were significantly lower in STNP-AdSC group compared with that in other groups. Simvastatin-conjugated nanoparticles enhanced the therapeutic effect of a small number of AdSC transplantation.

PANCREAS, LIVER, KIDNEY

T-1061

PLATELET-DERIVED MITOCHONDRIA DISPLAY EMBRYONIC STEM CELL MARKERS AND IMPROVE PANCREATIC ISLET BETA-CELL FUNCTION IN HUMANS

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Diabetes is a major global health issue, and the number of individuals with type 1 diabetes (T1D) and type 2 diabetes (T2D) increases annually across multiple populations. Research to develop a cure must overcome multiple immune dysfunctions and the shortage of pancreatic islet β cells, but these challenges have proven intractable despite intensive research over the past decades. In previous clinical trials for T1D, T2D, and other autoimmune diseases we demonstrated the safety and efficacy of Stem Cell Educator (SCE) therapy for re-educating autologous blood mononuclear cells to eliminate autoreactivity. In this study, we found that the percentage of platelets was increased after treatment with SCE therapy. Platelets display immune tolerance-associated markers such as the autoimmune regulator (AIRE) and program death ligand-1 (PD-L1) that can modulate the function and differentiation of monocytes/macrophages and other immune cells. Notably, platelets expressed embryonic stem (ES) cell- and pancreatic islet β-cell-associated markers that are encoded by platelet mitochondrial DNA (mitoDNA). Using freshly isolated human pancreatic islets, ex vivo studies established that platelet-releasing mitochondria can migrate to pancreatic islets via fibronectin/CD29, CXCR4/SDF-1 or other potential chemokines and be taken up by islet β cells, leading to the proliferation and enhancement of islet β-cell functions. Notably, pancreatic islet β cells can be reprogrammed to proliferate while maintaining good cell viability and restoring normal β-cell function. This study established that platelets not only display the critical molecules for inducing immune tolerance, but also carry the islet-specific transcription factor MAFA, the pancreatic progenitor-associated marker SOX9, and ES-related self-renewal markers (e.g., OCT4, SOX2, KLF4 and C-Myc). Thus, these approaches using platelets to protect and enrich islet β cells may mitigate safety and ethical concerns associated with approaches that involve the transplantation of insulin-producing cells or the viral/drug-induced transduction of pancreatic

cells. Targeting the mitochondria in platelets may revolutionize regenerative medicine with current stem cell approaches, leading to the development of novel treatments for human diseases.

T-1063

IN VITRO MAINTENANCE OF FUNCTIONAL NEPHRON PROGENITORS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Nephron progenitor cells (NPCs), the precursors of nephrons, can be generated from pluripotent stem cells. In conventional renal differentiation protocols, formation of nephron progenitors is a transient phase of differentiation and hence these cells cannot be maintained in progenitor state in culture. In this study, we established protocols for two-dimensional expansion of NPCs derived from human embryonic stem cells and induced pluripotent stem cells. After 15-fold expansion, NPCs retained the expression of NPC specific markers SIX2, CITED1, SALL1, as confirmed by immunocytochemistry. Further we showed that NPCs after cryopreservation can be culture expanded and retain the expression of NPC specific markers. Both culture expanded and cryopreserved NPCs are capable of nephrogenesis in organotypic cultures. Currently, we are evaluating NPCs expanded long-term in culture for the retention of NPC-specific properties. Long-term expansion of NPCs will provide a platform to get sufficient number of cells for kidney regeneration, drug screening and disease modelling of kidney disorders.

Funding Source: MERLN Institute for Technology Inspired Regenerative Medicine and Brightlands Materials Center Program on Additive Manufacturing: 3D Printing Biomedical Applications

T-1065

DERIVATION AND CHARACTERIZATION OF PANCREATIC DIFFERENTIATED MODY1-IPSCS

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MODY (maturity onset diabetes of the young) is a hereditary form of diabetes mellitus that manifests during childhood and adolescence eventually resulting in pancreatic β-cells dysfunction. MODY is a monogenic, autosomal dominant inherited disease that constitutes about 2-4% of all diabetic cases. Mutations in 14 genes are known to cause MODY, most of them encode transcription factors or other proteins essential for pancreatic development and function. Haploinsufficiency is the underlying genetic phenomena driving MODY disorders, since mutation in only one allele, in all the currently known genes involved in MODY, leads to the diabetic phenotype in heterozygous patients. MODY1 (5-10% of MODY cases) is caused by a mutation in the transcription factor hepatocyte nuclear factor 4 alpha (HNF4a), an important regulator of the development and function of the liver, kidney, colon, intestine and pancreas. In order to model MODY1 in human cells we generated induced pluripotent stem cells harboring mutated HNF4a by reprogramming somatic cells from patients with MODY1. Our study evaluated the effects of mutations in HNF4a on gene expression and differentiation of endodermal and pancreatic progenitor cells. We identified HNF4a targets that are expressed during pancreatic differentiation in WT cells but failed to be expressed in MODY1 cells. We have further found that HNF4a binding sites number and distribution along the promoter and HNF4a motif sequences affected the expression levels of HNF4a target genes in MODY1 cells. These features may partially explain the sensitivity for the decrease in HNF4a levels among its targets, underlying the mechanism of haploinsufficiency in MODY1 patients.

T-1067

TCF7L1 INDUCES BETA CELL DEDIFFERENTIATION IN DIABETES

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Diabetes affects approximately 29.1 million people in the United States, accounting for 9.3% of the population and costing an estimated $245 billion a year. Although the physical effects of diabetes are well documented, the genetic and molecular mechanisms responsible for the dysfunction/dedifferentiation of pancreatic beta cells during disease progression are not well described. Understanding those could lead to new advances in diabetes prevention and treatment. To this end, we are investigating the role of Tcf7l1 in the maturation and dedifferentiation of pancreatic beta cells. Tcf7l1 is a transcription factor in the Wnt signaling pathway that has been linked to a reversion of various adult cell types to an embryonic-like state when over-expressed. Analysis of a microarray of genes down regulated during beta cell maturation reveals that 26 of 66 down-regulated genes contain a Tcf7l1 binding site in their promoters. Bioinformatic pathway prediction suggests that overexpression of Tcf7l1 will repress the expression of beta cell core transcription factors.

Using immunostaining and qRT-PCR, we demonstrate that Tcf7l1 expression in β cells is a mirror image to the expression of these transcription factors: Tcf7l1 is downregulated during postnatal beta cell maturation and is re-expressed in islets from obese-diabetic mice. Further analyses using our Insulin-rtTA; TetO-Tcf7l1 (Tet-On) transgenic mice reveal that conditional expression of Tcf7l1 in mature beta cells results in a loss of a mature beta cell state in vivo, with concomitant glucose intolerance. Taken together, our results suggest that re-expression of Tcf7l1 under diabetic stress may have a causative role in deriving beta cell dedifferentiation.

T-1069

MOUSE PERICENTRAL HEPATOCYTES PRODUCE IGF-2 TO PROMOTE LIVER REGENERATION DURING CHRONIC INJURIES

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Liver regeneration happens after various types of injuries. Unlike the well-studied liver regeneration caused by partial hepatectomy, there is accumulating evidence indicating that liver regeneration during other injuries may result from some unknown mechanism. In this study, we found that insulin-like growth factor 2 (IGF-2) was drastically induced following the liver injuries caused by tyrosinemia or long-term treatments of carbon tetrachloride (CCl4). However, it was not observed during the acute liver injuries after partial hepatectomy or a single treatment of CCl4. We found that IGF-2 was mainly produced by the hepatocytes residing around the central vein of the liver lobule after the liver injuries present in either Fah-deficient mice or caused by chronic treatment of CCl4. Hepatocyte proliferations in vivo were significantly promoted by induced over-expression of IGF-2, which could be inhibited by linsitinib as an inhibitor of IGF-2 signaling. Proliferating hepatocytes in vivo responded to IGF-2 via both insulin receptor and IGF-1 receptor. IGF-2 also significantly promoted DNA synthesis of primary hepatocytes in vitro. More interestingly, in liver samples from patients with chronic liver diseases, significantly induced IGF-2 was also found to co-localize with the enhanced levels of glutamine synthetase in the region enriched with proliferating hepatocytes. Conclusion: IGF-2 was produced by perivenous hepatocytes to promote hepatocyte proliferation for tissue repair during some particular chronic liver injury induced liver regenerations, which is different from what occurs after partial hepatectomy.

T-1071

TRANSIENT EXPRESSION OF LIVER-RELATED CELL ADHESION MOLECULES IN HUMAN SKIN-DERIVED STEM CELLS AS TOOL TO IMPROVE THEIR LIVER ENGRAFTMENT

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Human skin-derived precursors (SKP) are somatic stem cells that reside in dermal skin throughout life harboring clinical potential. SKPs are immunologically-privileged due to their absence of MHC class II antigens HLA-DR and co-stimulatory molecules, providing a solid basis for their future clinical applications. We previously found that SKPs successfully engraft, survive and repopulate hepatic tissue in a transgenic murine model of liver deficiency (uPA+/+/SCID) and contribute to the increase in liver mass. Furthermore, SKPs secrete high levels of hepatocyte growth factor (HGF), a soluble molecule that plays a key role in liver regeneration and hepatocyte proliferation in vivo. These features render them plausible candidates for cell-based therapy for the treatment of various liver diseases. However, a major drawback that limits the use of cellular therapy for the treatment of liver disorders is the high percentage (> 70%) of transplanted cells that are cleared from the vascular bed 24h to 48h post transplantation. The mechanisms causing poor engraftment are thought to depend on the capacity of the transplanted (stem) cells to escape from apoptosis and sinusoidal clearance due to the lack of cellular attachment and vascular transmigration. Therefore, we hypothesized that liver engraftment of SKPs can be significantly improved by transient expression of liver-related cell adhesion molecules (CAM) such as epithelial cell adhesion molecule (EpCAM) using messenger RNA (mRNA) electroporation. First, we established the optimal electroporation parameters (pulse -type: square wave; -intensity: 500V; -time: 5ms) for SKPs using the Bio-rad Gene Pulser Xcell system and mRNA coding for green fluorescent protein (GFP). Next, we defined the kinetics of transient EpCAM expression in SKPs in vitro by flow cytometry and immunostainings and found high levels at 24h post electroporation, gradually decreasing over culture time. Finally, as a proof-of-principle, we evaluated the improved adhesive properties of EpCAM-positive SKPs in a cell adhesion assay together with primary human or hepatocytes and investigated their improved engraftment capacity upon transplantation in

the liver deficient FRG (Fah-/-/Rag2-/-/Il2rg-/-) mouse model.

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T-1073

WNT AND FGF PATHWAYS ARE REQUIRED FOR STEM CELL MEDIATED REGENERATION OF THE ADULT KIDNEY IN ZEBRAFISH

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In zebrafish, adult kidney injury can be repaired by stem cell-mediated nephron regeneration. This occurs by progenitor cell aggregation on kidney collecting ducts, cell condensation, and differentiation, leading to the insertion of new nephrons. We find that progenitor cell recruitment to sites of new nephron formation and differentiation/growth of new nephrons can be distinguished based on distinct growth factor requirements: FGF signaling is required for recruitment of progenitor cells to collecting ducts while Wnt signaling plays an essential role in new nephron differentiation/elongation. Expression of dusp6, a transcriptional readout of FGF signaling, is induced broadly at three days post injury (dpi) and becomes restricted to single cells at 5 dpi and ultimately to nephrogenic aggregates at 7 dpi. Chemical or genetic inhibition of FGF signaling completely prevents recruitment of nephron progenitor cells to nephron aggregates after acute injury. A survey of FGF ligands identified injury induced expression of fgf3, fgf4, fgf5, fgf10a, and the receptors FGFR2, FGFR4 and FGFRL1. Individual kidney progenitor cells and newly forming nephron condensates marked by expression of a Tg(lhx1a:GFP) reporter express the Wnt receptor frizzled 9b (fzd9b) and the canonical Wnt target gene lef1. Kidney injury induced multiple Wnt ligands including wnt9b in collecting duct epithelia and wnt4a in new nephron aggregates. Tg(TCFLef-miniP:dGFP) Wnt reporter expression revealed that new nephron aggregates are patterned by canonical Wnt signaling. High canonical Wnt signaling cells formed a single cell thick dome within cell aggregates and polarized to form rosettes with an apical constriction predicting the site of future tubule lumen formation. Nephron progenitor cell aggregates were also highly proliferative with an active zone of cell proliferation projecting away from high canonical Wnt+ cells. This proliferation was blocked using IWR1 and IWP2, which decreased the number of lhx1a+ nephron aggregates after injury and resulted in loss of rosette structures. Our results demonstrate an essential role for FGF and Wnt signaling in nephron progenitor recruitment, cell polarization, nephron condensate patterning, and nephron elongation during adult zebrafish kidney development and regeneration.

Funding Source: NIH/NIDDK, Harvard Stem Cell Institute

EPITHELIAL TISSUES

T-1075

MACROPHAGE-DEPENDENT COX-2/PGE2 SIGNALING PROMOTES EXPANSION OF CANCER STEM CELLS

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Cancer stem cells (CSCs) are known to enrich treatment-resistant cancer cells; however, the underlying mechanism remains largely unclear. Here we tested a hypothesis that the microenvironment protects CSCs and promotes CSC expansion in response to stress. In both human colorectal cancer patients and APCmin adenoma mouse model, we observed that the combined treatment with chemotherapy (capecitabine, a pro-drug of 5FU) and celecoxib, that inhibits cyclooxygenase-2 (COX-2), resulted in an increased overall survival (OS) rate compared to that of chemotherapy alone. Dissecting the cellular and molecular mechanism, we found that there was a significant reduction of CSC population following the combination treatment. Performing RNA-seq analysis on CSCs that received different treatments, we observed upregulation of prostaglandin E receptor EP2 and EP4 subtypes in the chemo-treatment group, accompanied with increased Akt3 and Wnt signaling, which was intriguingly suppressed in the Cox2 and combination treatment groups. Furthermore, we found that Tumour-associated macrophages (TAMs) (CD11c+,CD11blo,hi, MHCIIhi by flow or CD68+ using immunostaining) formed a microenvironment to protect CSCs (CD44hiCD24lo), with an upregulation of COX-2/PGE2 pathway and increased Akt and Wnt signaling. Intriguingly, chemotherapy promoted TAMs clustering surrounding CSCs and an increase in these signals as described, which, however, were suppressed by the combined treatment or by macrophage depletion using Clodrosome. Using immunostaining assay, we further detected heterogeneity of adenoma with Cox2pos region and Cox2low-negative region; and only the former was sensitive to the combined treatment. Strikingly, PD-L1 was detected only in the Cox2low-negative region, suggesting a potential role of TAMs and COX-2/PGE2 signaling in inhibiting PD-L1 expression. Taken together, TAMs forms a microenvironment to protect CSCs and

to promote CSC expansion via COX-2/PGE2 pathway and Akt-Wnt signaling in response to chemotherapy, as well as to regulate immune-checking, thus efficiently controlling the tumor growth.

T-1077

AMNIOTIC FLUID STEM CELL DERIVED EXOSOMES RESCUE HYPOPLASTIC LUNGS IN A FETAL RAT MODEL OF CONGENITAL DIAPHRAGMATIC HERNIA

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Lung hypoplasia plays a major role in the outcome of neonates with congenital diaphragmatic hernia (CDH). Recently, amniotic fluid stem cells (AFSC) were shown to rescue lung hypoplasia via an undetermined paracrine effect. We aimed to investigate whether this beneficial effect was due primarily to AFSC exosomes. To test this hypothesis, we isolated and characterized AFSC exosomes, and applied them to an in-vitro and an ex-vivo model of lung injury. AFSC exosomes: isolated via ultracentrifugation from AFSC conditioned medium (CM), quantified by protein content (BCA assay), and characterized by size (Nanoparticle Tracking), surface marker expression (CD9, CD63, CD81, Hsp70 via Western blot), and morphology (transmission electron microscopy). In-vitro: primary epithelial cells were harvested from E14.5 fetal lungs whose dams received nitrofen (100mg) at E9.5 to induce lung hypoplasia. EpCAM+ cells were isolated using magnetic beads, cultured for 5 days in bronchial epithelial cell growth medium, and treated with: i. growth medium alone; ii. AFSC-exosomes (200ug/mL); iii. AFSC-CM; iv. AFSC-exosome-free CM. Lungs from fetuses not exposed to nitrofen served as control. Groups were compared for proliferation (5'EdU) and apoptosis (live/dead assay) using one-way ANOVA (Tukey post-test). Moreover, protein and RNA cargo was labelled with Exo-Glow™, added to epithelial cells, and tracked under microscopy. We found that the detrimental effect on cell proliferation and apoptosis was rescued by AFSC-exosomes and AFSC-CM, but not by AFSC-exosome-free CM. AFSC-exosome RNA was endocytosed within 15min, whereas exosome proteins within 1h. Ex-vivo: E14.5 lungs were harvested and treated for 72h as described above (i-iv). Nitrofen administration resulted in severe lung hypoplasia. We found that lungs treated with either AFSC-CM or AFSC-exosomes had terminal bud density and surface area similar to normal control. Conversely, explants treated with AFSC-exosome-free CM failed to rescue lung hypoplasia. Exo-Glow™ stained AFSC-exosomes migrated into the explant as visualized by two-photon excitation microscopy. This study shows for the first time that AFSC-exosomes are able to rescue lung hypoplasia in fetal rats. Stem cell secreted vesicles could represent a promising cell-free therapy for babies with CDH.

T-1079

TRANSCRIPTIONAL AND FUNCTIONAL DIFFERENCES IN MOUSE INTESTINAL CRYPTS DURING AGING

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Aging is associated with a decline in intestinal function characterised by a reduced capacity to take up nutrients, susceptibility to infection and old age colitis. Moreover, the incidence of colorectal cancer dramatically increases with age. Age related changes in other tissues, such as muscle and blood, have been attributed to alterations within the stem cells and/or their associated niches. However, whether similar changes occur within intestinal stem cells (ISCs) and their supporting niche environment is currently unclear. To study the effect of aging within intestinal crypts, we performed morphological and immunohistochemical characterisation of the intestinal epithelium during homeostasis and tissue regeneration following injury in young versus old mice (2 vs 22 month old). We observed significant changes in the cellular composition of crypts and villi with aging, including an enlargement of the crypt compartment and increased number of ISCs. Furthermore, we determined that the intestinal epithelium of aged animals is significantly more vulnerable to damage induced by the chemotherapeutic agent 5-FU. In order to define the underlying molecular mechanisms of these age related changes, we isolated both ISCs and Paneth cells, which have been identified as a key epithelial niche cell population, from different age groups (2 and 22 month old) and performed RNA sequencing. Differential expression and principal component analysis revealed that the molecular signature of young and old ISCs were distinct (446 genes), in contrast to Paneth cells which were similar (30 genes). The functional capacity of ISCs was examined by studying the ability of isolated ISCs to establish organoids. ISCs from old animals produced less organoid colonies compared to ISCs from young mice irrespective of supplementation with young or old Paneth cells at the time of plating. Overall, our work suggests that age-related changes are present in intestinal crypts with transcriptional differences observed in ISCs but not Paneth cells.

T-1081

NEURAL CREST CELLS CONTRIBUTE TO PITUITARY DEVELOPMENT

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The mechanism for regeneration of the pituitary gland is still unclear. Our recent observation indicated that a part of cells invading the pituitary express p75NTR and/or Sox10, markers of neural crest cells (NCCs). NCCs are multipotent cells arising in the embryonic ectoderm at the margins of the neural tube. Moreover, NCCs are known to migrate and invade the various tissues. Although most of them transformed into residents of respective tissues, a part of them are known to remain as somatic stem cells. Here, we attempted gene tracing analysis of NCCs using P0-Cre/CAG-CAT-EGFP mice to clarify the contribution to pituitary development. Gene tracing analysis revealed that a small number of GFP-positive cells invaded the primordium of the anterior pituitary on embryonic day 9.5 (E9.5). Subsequently, a large number of GFP-positive cells invaded the developing pituitary through Atwell's recess that receives primitive portal vessels. Double-immunostaining with Cre-recombinase and GFP revealed that a number of Cre/GFP-double positive cells localized in the pituitary gland on E14.5, although GFP-positive cells inside the primordium on E9.5 were Cre-negative. These data indicated that different types of GFP-positive cells invaded the pituitary gland by a stepwise manner. Next, we characterized GFP-positive cells in the embryonic anterior pituitary. Triple-immunostaining with GFP and the pituitary stem/progenitor cell markers, SOX2 and PROP1, revealed that some GFP-positive cells acquired the characteristics of pituitary stem/progenitor cells. Moreover, double-immunostaining with GFP and pituitary hormones demonstrated that GFP-positive cells differentiated into terminally differentiated cells. Finally, we performed double-immunostaining with GFP and NG2, one of the pericyte markers, because a large number of GFP-positive cells invade through Atwell's recess. The result showed that NG2/GFP-double positive cells localized in the anterior pituitary, indicating that GFP-positive cells also contribute to pituitary vasculogenesis. Taken together, present study revealed that NCCs invade the anterior pituitary gland by a stepwise manner, and transform into the pituitary stem/progenitor cells to supply hormone-producing cells in addition to pericytes.

T-1083

HORMONE INDUCE TRANSDIFFERENTIATION OF MAMMARY LUMINAL CELLS TO BASAL STEM CELLS

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The mammary gland is a bi-layered epithelium composing basal cells and luminal cells. Mammary stem cells capable of generating both cell types are resided in the basal layer, whereas luminal cells are believed to be unipotent throughout development. Here, by lineage tracing, we discover that a small population of luminal cells exhibits plasticity by acquiring stem cell properties during pregnancy. These luminal-derived mammary stem cells (Ld-MaSCs) display basal characteristics and persist into the involuted mammary gland. In ovariectomized (OVX) mice, hormonal stimulation can potently induce the formation of Ld-MaSCs. Upon further tracing, Ld-MaSCs are able to proliferate and contribute to both basal and luminal cells in tissue homeostasis and subsequent rounds of pregnancy. Isolated Ld-MaSCs can reconstitute a new mammary gland in transplantation assays. Our data uncover an unexpected potential of luminal cells that can regain multipotency in physiological and pathological conditions, providing new insights into the association of hormone and breast cancer.

T-1085

SPATIOTEMPORAL DYNAMICS OF INTESTINAL STEM CELL SELF-RENEWAL AND DIFFERENTIATION

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Stem cells possess unique self-renewal and differentiation capabilities. The dynamic behaviors (i.e. self-renewal vs. differentiation) of stem cells are collectively determined by their intrinsic genetic programs and epigenetic states, as well as extrinsic cues supplied by the stem cell niche. Lgr5 Intestinal stem cells (ISCs) reside at the bottom of intestinal crypts, and receive niche signals including Wnt, Notch and BMP inhibitors. By using a defined in vitro organoid culture and controlled ISC self-renewal and differentiation system, we found that the fate determination of Lgr5 ISCs is a spatiotemporally dynamic process, instead of a simple combination of different niche signals, which provides another layer of cell fate regulation. Specifically, at different time points,

ISC derived epithelial cells (e.g. TA cells and progenitor cells) change their cellular states (e.g. genetic and epigenetic states) while they migrate to different locations along the crypt-villus axis, thus receiving multiple micro-environmental signals from underlying mesenchyme as well as from neighboring epithelial cells. The dynamic response of the cells is then influenced by the temporal state of cells and spatial restricted signals they received, which collectively determines the cell fate. We discovered the importance of applying temporal control of signaling pathways, especially Wnt and Notch signals in regulating ISC differentiation, which greatly influence the fate determination of secretory progenitors towards goblet, Paneth or enteroendocrine cells. In addition, via small molecule screening, we identified other signaling pathways that play important roles in ISC differentiation, such as EGFR/MEK, Tgf-β and epigenetic regulation. Through emulating the process of ISC self-renewal and differentiation, we established a platform to generate high purity ISC and their progenies, including high purity (near 100%) enteroendocrine cells which was not previously achieved.

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T-1087

HYPOXIA-CONDITIONED ADIPOSE MESENCHYMAL STEM CELLS PREVENT IRRADIATION-INDUCED SALIVARY HYPOFUNCTION

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Hypoxic conditioning of MSC has been introduced for the promotion of therapeutic effects; however, the interaction of hypoxia (HPX) and MSC function on radioprotection has not been well described. Herein, we investigated the effects of hypoxic conditioning on the radioprotection of human adipose mesenchymal stem cells (hAdMSCs) against irradiation (IR)-induced salivary hypofunction and the modes of paracrine action by key factors secreted from hAdMSCs activated by hypoxia. A three-dimensional (3D) IR model to mimic the natural microenvironment was employed for in vitro experiments. Human parotid gland epithelial cells (hPECs) were organized to form 3D acinar-like spheroids on GFR-Matrigel, then irradiated to induce cellular, structural, and functional damages. The irradiated 3D spheroids were subsequently co-cultured with hAdMSCs that had been preconditioned under either normoxia (NMX, 20% O2; hAdMSCNMX) or hypoxia (HPX, 1% O2; hAdMSCHPX). IR elicited structural alterations and reduced the expression of salivary structural maker genes and proteins, but the damage was alleviated by both hAdMSCHPX and hAdMSCNMX. hAdMSCHPX led to greater preservation of epithelial integrity and acinar secretory function of α-amylase in response to agonists relative to hAdMSCNMX. Microarray revealed that hAdMSCHPX significantly enhanced paracrine secretion, including anti-apoptotic or angiogenic factors relative to hAdMSCNMX. Among secreted factors, we found that FGF10 exerted a significant anti-apoptotic effect in a dose-dependent manner that was dependent on FGFR-PI3K-p53 signaling. IR-induced cell death of hPECs in 3D spheroids was attributed to the p53-mediated apoptotic pathway. Co-culture with hAdMSCHPX suppressed p53 activation via the PI3K–protein kinase B (AKT) pathway, in which a downstream target, MDM2, subsequently downregulated p53 activation along with a decrease in a negative regulator, PTEN. In vivo transplantation of either hAdMSCHPX or hAdMSCNMX into irradiated salivary glands of mice could restore IR-induced salivary hypofunction in which hAdMSC-released FGF10 contributed to tissue remodeling.

STEM CELL NICHES

T-1089

PROTEOME RESPONSE OF DPSCS TO TREATMENT WITH EXOGENOUS FGF8

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The aim of the study was to examine differential protein expression in adult human dental pulp cells in response to short- and long- term treatment with FGF8 in order to assess its value as a therapeutic agent for the purposes of pulp regeneration. Intact human wisdom teeth were obtained from healthy patients between the ages of 18 and 40 following the acquisition of written consent. Cell cultures were maintained at standard culture conditions supplemented with 10% FBS. Upon reaching third passage, samples run in triplicates were subjected to short- (24h) and long-term (10 days) treatment with 10ng/ml human recombinant FGF8. Following 2D DIGE analysis differentially expressed proteins were identified by MALDI TOF/TOF MS. These belonged to gluconeogenesis (triosephosphate isomerase), cellular structure and motility (gelsolin, moesin, caldesmon, zyxin, CAPG, vimentin, prelamin A/C), inflammation (Leukotriene A-4 hydrolase), protein biosynthesis, trafficking and degradation (Golgi-associated PDZ and coiled-coil motif-containing protein, COP9 signalosome complex subunit 5, Heat shock 70 kDa protein 1A, chloride intracellular channel protein 4), cell cycle and DNA repair (14-3-3 protein beta/alpha; X-ray repair cross-complementing protein 5), ECM maturation (protein disulphide isomerase, lysyl hydroxylase, CAPG). In summary, the proteomic approach has yielded novel information about the effect of FGF8 on cells of adult dental pulp with identified proteins being linked to

the regulation of actin cytoskeletal rearrangements, migration and odontogenic differentiation, consistent with what has been reported about the role of FGF8 in the context of embryonic tooth development and the neural crest origin of dental pulp cells. FGF8 is likely to be able to instigate pulp cell migration and differentiation in response to pulp injury in an appropriate model organism, however further studies need to be conducted to validate its therapeutic potential.

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T-1091

LET-7 REGULATES ADULT NEUROGENESIS THROUGH POSITIVE REGULATION OF AUTOPHAGY

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During adult neurogenesis, neural stem cells (NSCs) reside in the subventricular zone (SVZ) where they continuously give rise to neuroblasts that migrate along the rostral migratory stream (RMS) into the olfactory bulb (OB). There they radially migrate into the cellular layers of the OB, differentiate to mature interneurons and integrate into the existing neuronal circuitry. Despite extensive research, molecular mechanisms underlying radial migration, maturation and integration of new-born neurons are still not fully elucidated. Recently microRNAs (miRNAs) have been suggested to play an important role in the regulation of adult neurogenesis. miRNAs are small, non-coding, single stranded RNAs that regulate mRNA activity by associating with a protein called Argonaute2 (AGO2). To identify miRNAs with a potential role in adult neurogenesis, we conducted Argonaute2-immunoprecipitation, followed by small RNA sequencing (AGO2-RIPseq) on new-born OB neurons. Using this approach, we identified the highly conserved miRNA let-7 as the most abundant miRNA family in new-born neurons. Interestingly, knockdown of let-7 in new-born neurons impaired their radial migration into the outer OB layers and prevented them to fully mature. This phenotype was accompanied by a decrease in autophagic activity. Moreover, activation of autophagy in new-born neurons lacking let-7, restored their ability to radially migrate into the cellular layers of the OB but not their maturation. Taken together, our results demonstrate a novel role for let-7 in adult neurogenesis and reveals a miRNA-dependent link between autophagy and adult neurogenesis.

T-1093

BIVALENT ROLE OF FGF2 IN MOUSE SPERMATOGONIAL NICHE

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Although previous study identified fibroblast growth factor 2 (FGF2) as another self-renewal factor for spermatogonial stem cells in vitro, it's function in the germline niche remains to be elucidated. Therefore, we investigated the function of FGF2 in mouse germline niche. Although FGF2 and GDNF exhibited differential expression dynamics under physiological condition, both are upregulated during regeneration, suggesting the contribution to testicular regeneration. Indeed, both molecules induced spermatogonial proliferation by testicular administration via drug delivery system. However, FGF2 upregulated RARG in undifferentiated spermatogonia and downregulated retinoic acid (RA) metabolizing enzyme gene, in the germline niche, suggesting that FGF2 facilitate spermatogonial differentiation via RA signal. Taken together, our research suggested that FGF2 is a bivalent factor in the mouse spermatogonial niche, facilitating both self-renewal and differentiation of undifferentiated spermatogonia, providing new insights into the regulation of undifferentiated spermatogonia by FGF2.

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T-1095

IN VIVO LABELING OF MESENCHYMAL DERIVATIVES ENABLES THE MOLECULAR CHARACTERIZATION OF BONE MARROW SINUSOIDAL CELLS

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Mesenchymal stem cells (MSCs) are multipotent cells residing in the bone marrow (BM) and other tissues.

To enable identification and tracking of MSCs in vivo, we established a CD73-EGFP reporter mouse which facilitates enrichment of MSC-like cells with typical marker expression and tri-lineage differentiation potential. EGFP+ mesenchymal progenitor cells were apparent in fetal and adult bone and showed a perivascular distribution in several organs. Primary cultures from BM, epiphysis and white fat gave rise to fibroblastic colonies (CFU-Fs) containing EGFP+ cells with the ability to differentiate into mesenchymal lineages. Furthermore, we could observe elongated EGFP+ cells at the endosteal site of adult cortical bone. We isolated these bone lining cells (BLCs) by enzyme treatment of bone fragments and they gave rise to CFU-Fs after several days in culture. They revealed expression of typical MSC markers and multipotent differentiation potential in vitro. Single cell cultures of BLCs generated CFU-Fs at high frequency and also showed tri-lineage differentiation potential, proving on clonal level that EGFP+ cells are true stem cells. An unexpected feature of the mouse model was labeling of endothelial cells in the BM. These EGFP+ cells expressed typical endothelial markers and had the ability of tube formation in vitro. Stainings revealed that EGFP expression was exclusively found on the sinusoidal endothelial population allowing for distinction from EGFP- arteriolar endothelial cells. Comparative RNAseq and subsequent gene set enrichment analysis demonstrated that sinusoidal cells showed a mesenchymal signature and higher expression of E-selectin and Vcam-1 compared to arteriolar endothelium. Taken together, our mouse model identifies MSCs and specifically sinusoidal cells within the bone marrow endothelial compartment. This allowed for direct visualization and molecular characterization of these important components of the HSC niche.

T-1097

MULTIPHOTON FABRICATION OF FUNCTIONALIZED PROTEIN MICROPATTERNS – AN IN VITRO MODEL FOR STUDY ON CELL-MATRIX INTERACTIONS IN HUMAN MESENCHYMAL STEM CELLS

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Micropatterning technique is a powerful tool in cell niche studies. Multi-photon-based micropattern fabrication is a promising technology able to incorporate bioactive molecules into process as well as precisely control the geometry and stiffness of micropatterns fabricated. In the present study, we aim to engineer fibronectin-functionalized micropatterns with different stiffness based on multiphoton fabrication technique, and test the validity of this versatile tool on investigation of the cell-matrix adhesion formation and matuaration in human mesenchymal stem cells (hMSCs). Firstly, the fibronectin-functionalized protein micropatterns were fabricated by photo-crosslinking Bovine Serum Albumin (BSA) (100 mg/ml) and various concentrations of fibronectin (0 μg/ml, 50 μg/ml, 150 μg/ml and 350 μg/ml) in the presence of photosensitizer, Rose Bengal (RB) (0.1%, w/v), which was activated by femto-second laser with output power of 60 mW at wavelength of 800 nm. Then the cross-linked fibronectin was verified by immunofluorescence staining. Afterwards, fibronectin-functionalized protein micropatterns with different dimensions (length × width × height: 1 × 1 × 5 μm, 3 × 1 × 5 μm and 10 × 1 × 5 μm) were fabricated as described above using different region of interest (ROI). Finally, hMSCs were cultured on these micropatterns for 3 days to determine the maturation of 3D-matrix adhesions and fibrillar adhesions using immunofluorescence staining. Results show that immobilization of fibronectin by multiphoton photo-crosslinking on the protein micropatterns is dose-dependent of the initial amount of fibronectin incorporated. Fibronectin-functionalized micropatterns with longer length could induce the maturation of cell-matrix adhesions in terms of formation of fibrillar adhesion (FBA) in hMSCs. The current platform presents a good in vitro model for cell niche studies.

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EYE AND RETINA

T-1099

DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO CONE PHOTORECEPTORS FOR RETINAL DEGENERATIVE DISEASE MODELING

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Macular degenerations are retinal disorders affecting millions of people worldwide. Photoreceptors (PRs), the specialized cells involved in phototransduction, progressively degenerate leading to vision loss. Retinal degeneration is observed in many ciliopathies such as Meckel-Gruber (MKS), Retinitis Pigmentosa and Bardet-Biedl (BBS) syndromes. Currently, there isn't any model available to study these diseases, as well, there is no treatment to delay, stop or reverse them, making retinal degenerations incurable. We recently reported a method to differentiate human Pluripotent Stem cells (hPSc)

into cone PRs. My goal is to use this method, patient-specific iPSc, and gene editing technologies to model retinal diseases. We identified the causing mutations and generated induced Pluripotent Stem cells (iPSc) from BBS (BBS10) and MKS (TMEM67) patients as well from healthy volunteers. IPS ctrl cells maintained for 60 days in differentiation media on Matrigel, spontaneously organize in a polarized, multi-layered tissue with more than 80% of cells expressing cone PR markers. These "cones-sheets" shows the formation of Inner Segment (IS), Connecting Cilium (CC) (positive for RPGR and RP2), and Outer Segment (OS) (positive for PNA and S-OPSIN). MKS PRs were characterized by the swelling of the outer segments (PNA staining) and the formation of large cytoplasmic aggregates containing RPGR. BBS PRs had poor OS formation and a cytoplasmic RPGR localization confined around the nuclei, as the migration to CC was totally lost. WB analysis supported these results showing decreased S-OPSIN and α-acetylated tubulin levels. Finally, we decided to inactivate BBS10 and TMEM67 in our ctrl iPSc using CRISPR/Cas9 technology to confirm our observations. This work is a proof of principle that retinal diseases modeling using patient-specific iPSc is possible and it also opens new insights towards the cure of macular degenerations.

Funding Source: The Foundation Fighting Blindness Canada (FFB Canada); Antoine-Turmel Foundation; HMR Foundation; University of Montreal

T-1101

COMPARISON OF DEVELOPMENTAL DYNAMICS IN HUMAN FETAL RETINA AND HUMAN PLURIPOTENT STEM CELL DERIVED RETINAL TISSUE

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Retinal degenerative diseases such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP) are incurable blinding conditions, severely impacting quality of life and affecting millions of people. Finding efficient treatment for these devastating diseases is among the greatest unmet clinical needs. Retinal replacement strategy can bring a piece of healthy mutation-free human retina into a patient's eye to replace degenerated retinal tissue. The only donor tissue, which was demonstrated to work in animals with retinal degeneration as well as in RP patients is human fetal retinal tissue. However, fetal retina has limited availability and ethical constraints. The 3D retinal tissue (retinal organoids) derived from human pluripotent stem cells (hPSCs) shares many similarities with human fetal retina and may be an excellent replacement of fetal retinal tissue in retinal transplantation experiments. The aim of this study is to find similarities and differences in distribution and expression of molecular markers in human fetal retina and in hPSC-derived 3D retinal tissue. To assess the similarities between fetal retina and hPSC derived 3D retinal tissue we used immunohistochemistry and RNA-seq methods. Preliminary results showed high correlation in gene expression profiles between human fetal retina and hPSC derived 3D retinal tissue. Immunohistochemical profiling of developing human fetal retinal tissue 8 -16 weeks showed strong expression of retinal pigment epithelium (RPE) markers (EZRIN, Beta-catenin), retinal progenitor markers (OTX2, CRX, PAX6), photoreceptor marker (RCVRN), amacrine marker (CALB2) and ganglion marker (BRN3B). Our study will streamline the development of hPSC-3D retinal tissue technologies aimed at repairing and replacing human retina affected by degeneration and causing irreversible blindness.

T-1103

GENERATION OF A VSX2::EGFP REPORTER CELL LINE TO DECIPHER THE MECHANISMS OF HUMAN RETINAL DIFFERENTIATION IN STEM CELL CULTURES

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Inherited retinal degenerations are the major causes of incurable vision impairment and blindness. The lack of faithful models represents a challenge to study disease mechanisms and to develop effective treatments. Recent advances in stem cell-based vision research have brought hope to save and restore vision. Retinal cultures have been generated from human embryonic stem cells and induced pluripotent stem cells through in vitro retinal differentiation, and the retinal cultures are being evaluated for the applications in disease modeling, drug discovery and testing, and potential cell replacement therapy. For these applications, the retinal cultures need to be sufficiently mature, retinal differentiation procedures need to be highly reproducible and scalable. Deeper understanding of retinal differentiation in stem cell cultures is a prerequisite. In mouse retinal development, homeodomain transcription factor VSX2 is a specific marker for neuroretinal progenitors. In a human retinal differentiation system we have developed recently, VSX2-positive progenitors spontaneously organize into apical convex epithelium, which generates mini retinae in long-term cultures. Therefore, generating VSX2-positive progenitors and tracking their cell behaviors are useful to decipher the mechanisms of human retinal differentiation. In this study, we describe the generation of a VSX2::EGFP reporter cell line using CRISPR/Cas9 technology. 2A-EGFP cassette was targeted to VSX2 locus immediately before the stop codon in H1 hESCs. EGFP reporter recapitulated endogenous VSX2 expression. Importantly, imaging of

EGFP reporter in retinal cultures confirmed our previous findings that VSX2 positive progenitors spontaneously organize into apical convex epithelium. Thus, we have generated a VSX2::EGFP reporter cell line that has multiple applications in modeling human retinal differentiation and disease.

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T-1105

PRECLINICAL SAFETY STUDIES OF HESC-DERIVED RPE CELLS INCLUDING TUMORIGENICITY, BIODISTRIBUTION AND GENOMIC STABILITY: A STEP TOWARDS TREATMENT OF AGE-RELATED MACULAR DEGENERATION

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Dry Age-related Macular Degeneration (AMD) is the leading cause of blindness in people aged 65 and older in industrialized countries. This disease is untreatable at present and its main feature is the degeneration of Retinal Pigment Epithelium (RPE), leading to a progressive photoreceptor degeneration and vision loss. We have recently established a novel xeno-free and robust hES cell in vitro differentiation protocol using human recombinant laminin 521. The hES-RPE cells have been transplanted into a large-eyed preclinical model, the rabbit, with promising functional results. Exhaustive characterizations have demonstrated that the cells obtained by this protocol share the main morphological, histological, physiological and functional features with native RPE cells, which makes these cells a good candidate for a possible treatment. Since the obtained cells have been differentiated from hES cells, it cannot be ignored that a fraction of undifferentiated and possibly tumorigenic cells may still reside in the product. Therefore, before these cells could be considered for further clinical applications, exhaustive tumorigenicity tests on mice and other safety studies have to be performed in order to assess the purity and safety of the final product. To perform the tumorigenicity studies, several groups of NOG mice have been subcutaneously injected, with increasing number of hES cells (from 10 to 1 million cells) to establish how many lingering cells could generate a tumorogenic growth. In parallel, 10 million cells from three time points along the differentiation protocol have been injected. 10/10 mice injected with 1 million of hES cells and 8/10 mice injected with 10.000 hES cells developed teratomas, whereas no teratoma formation has been observed in any of the mice injected with 10 million of mature hES-RPE cells. Furthermore, we have undertaken karyotyping, genotyping and whole genome sequencing of the original hES cells and the mature hES-RPE cells to evaluate the possible introduction of harmful alterations of the genome. Finally, bio-distribution of transplanted hES-RPE cells has been undertaken in transplanted rabbits and mice. All these safety tests are showing a positive outcome, which would promote our hES-RPE cells to take a step forward into further clinical trials.

NEURAL DEVELOPMENT AND REGENERATION

T-1109

BENEFICIAL EFFECT OF HUMAN EMBRYONIC STEM CELL-DERIVED NEURAL PRECURSORS COMBINED WITH A HYDROGEL IN SPINAL CORD INJURY REPAIR

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Stem cell-based therapy is a promising approach for the treatment of various central nervous system disorders including spinal cord injury (SCI). Neural stem and progenitor cells (NSCs) have been shown useful for transplantation therapy in SCI due to their ability to provide a source of cells for replacement, as well as trophic support. In order to improve the efficacy of transplantation, biomaterials are being used together with cells. Self assembly peptide hydrogel scaffolds are a class of biomimetic materials consisting of structure forming peptide sequences that closely mimic the extracellular matrix. In this study, the motor recovery, tissue regeneration and the degree of inflammation was evaluated after transplantation of human NSCs and a self-assembling nanofibrous hydrogel into a subacute model of rat SCI. Male Wistar rats were subjected to a spinal cord injury by contusion and divided into four groups: SCI only, hydrogel treatment, NSCs transplant and hydrogel implant together with NSC transplant. 200 µg of an Fmoc-DIKVAV hydrogel was injected into the lesion epicenter one hour after the SCI and 7 days after SCI, 2x 105 NSCs were transplanted centrally into the lesion site. The Basso, Beattie and Bresnahan (BBB) locomotor rating scale was used to assess motor recovery before surgery, two days after and weekly up to six weeks after transplantation. At day 42, the BBB scores were 8.5±2.3 for the SCI group, 9.0±3.1 for the NSC group, 14.0±3.6 for the hydrogel group and 9.1±3.8

for the NSC and hydrogel group. All the groups showed improved BBB scores relative to the vehicle alone (positive control), but only the hydrogel group showed significantly higher BBB scores when compared with the vehicle. The rats transplanted with NSCs showed reduced cavities and the presence of increased cellular material at the injury site, indicating the presence of transplanted cells. To quantify the glial scar, the NSCs fate and neural regeneration, and the inflammatory response, flow cytometry analysis was performed with various antibodies. The data shows that the transplanted groups showed improved neural regeneration, an increase in oligodendrocytes, inflammation and glial scar in the transplanted groups. This work illustrates the importance of using biomaterials as scaffolds for stem cell transplantation in spinal cord injury.

Funding Source: CNPq, FINEP, MCTI, and Stem Cell Research Institute

T-1113

CHARACTERIZATION OF IMMATURE NEUROBLASTS PRESENT IN THE ADULT MOUSE SUBVENTRICULAR ZONE

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We have developed a fluorescent-activated cell sorting method to isolate the different progenitors of the subventricular zone (SVZ) using a combination of 3 markers LeX, EGF fluorescent ligand and CD24 (Daynac et al. Stem cell Research 2013). In the present work, we performed microarrays from the RNA of the different isolated SVZ populations: CD24-LeX+EGFR+ (activated neural stem cells), CD24-Lex+EGFR+ (transit amplyfing cells), CD24+ EGFR+ (immature neuroblasts, iNBs) and CD24+ EGFR- (mature neuroblasts). Our transcriptomic analysis revealed that these 4 populations belong to 4 distinct clusters with specific characteristics providing thus a validation of our cell sorting method. We then compared the in vivo neurogenic potential of purified iNBs from actin::GFP mice just after cell sorting versus after 2 weeks of culture in physiological oxygen conditions by intracranial injections in wild-type recipient mice. Our results evidenced an unexpected plasticity of iNBs and highlight the interest to target these cells for the development of regenerative therapies of the damaged brain.

T-1115

PRECLINICAL DEVELOPMENT OF AN AUTOLOGOUS INDUCED PLURIPOTENT STEM CELL-DERIVED CELL THERAPY FOR PARKINSON'S DISEASE

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Several groups around the world are rapidly approaching clinical trial of stem cell-derived cell therapies for Parkinson's disease (PD). PD is an attractive target for a cell replacement therapy because the symptoms are due primarily to the loss of a single cell type which produces dopamine and no therapy exists which stops the progressive degeneration of the remaining dopaminergic (DA) neurons. Additionally, plans for clinical trials build upon the knowledge gained from previous studies using fetal tissue performed in the 1980s and 1990s. The last five years have brought dramatic improvements in the protocols used to produce authentic A9 DA neurons from pluripotent stem cells bringing clinical study within reach. Allogeneic approaches propose the use of immunosuppressants for up to one year after cell transplantation. We are developing an autologous, iPSC-derived cell therapy for PD which will not require the use of immunosuppressants. We have derived ten extensively characterized iPSC lines under IRB approval using a non-integrating reprogramming method. As part of our preclinical development, we have characterized the reproducibility of our differentiation protocol with all ten cell lines in vitro. In addition, we have performed efficacy and biodistribution and safety studies out to 9 months in a nude rat model of PD using four patient lines. 6-OHDA lesioned rats treated with patient-derived DA neurons decreased amphetamine induced rotations after cell transplantation indicating a restoration of dopamine signaling within the lesioned hemisphere. In our safety study, no tumors were observed (N=15 for each cell line) through 9 months. Whole-genome gene expression analysis was performed on all cell lines after 13 and 25 days of differentiation and compared to in vitro and in vivo cell characterization. We have identified modules of genes which are up regulated in cultures with a high yield of A9 DA neurons. We aim to develop a bioinformatic assay, similar to Pluritest, which can replace the use of animal studies to predict the in vivo efficacy of cell preparations from novel iPSC lines. Such a test will lower the current hurdles to the development of autologous iPSC-derived cell therapies.

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T-1117

"DEVELOPMENTAL WINDOW" OF PQQ-INDUCED MITOCHONDRIAL BIOGENESIS DURING HIPSC NEURONAL DIFFERENTIATION

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Pharmaceuticals which induce mitochondrial biogenesis may affect neural stem cell (NSC) development. Pyrroloquinoline quinone (PQQ) is a well-studied compound inducing biogenesis of mitochondria. In this study we investigated the effect of PQQ on viability, proliferation, mitochondrial biogenesis and differentiation potential in human induced Pluripotent Stem Cells (hiPSC) at different stages of neural differentiation: neural stem cells (NSC), early neural progenitors (eNP) and neural progenitors (NP). Induction of mitochondrial biogenesis by PQQ at three stages of neural differentiation was evaluated at DNA, mRNA and protein level. Our results indicate that sensitivity to PQQ is dependent on its concentration and on hiPSC stage of development. The toxic effect of PQQ was observed only at highest tested dose, selectively at NP stage. Changes in NRF1, TFAM and PPARGC1A gene expression were observed at all developmental stages, but only at the eNP stage they correlated with the statistically significant increase in the mtDNA copy number and higher levels of SDHA and COX-1 proteins. The observed effect was independent of the antioxidant properties of PQQ. In addition, a strong induction of GFAP, with down regulation of MAP2 gene expression upon PQQ treatment was observed. Thus, the existence of a "developmental window" of eNP cells for PQQ-induced mitochondrial biogenesis is proposed. Our study indicates the possibility of in vitro shifting cell differentiation in the favor of glia, but more research is needed at this point.

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T-1119

FUNCTION OF REELIN AND GM1 GANGLIOSIDASE DURING NEURAL DIFFERENTIATION OF ADIPOSE STEM CELLS

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The incidence of neurological disease in the population is of growing concern, however the mechanisms underlying the etiology of a host of neurological diseases remain ambiguous. Current evidence implicates disturbances of signalling pathways during neurodevelopment as a causative factor, however a robust model for studying neurodevelopment is required. This study aimed to utilize the neural differentiation of mesenchymal stem cells as a model for neurodevelopment, in order to analyse the expression and function of the proteins Reelin and GM1 gangliosidase, the malfunction of which are correlated with an increased risk of schizophrenia, Alzheimer's Disease, and Parkinson's Disease. Cell samples and secretions were collected at various time points during neural differentiation of adipose stem cells, and underwent proteomic analysis via shotgun LC-MS/MS, BN-PAGE, Western blotting, and Bioplex multiplex immunoassay. Reelin and proteins pertinent to Reelin signalling and neuronal migration were detected, whilst GM1 gangliosidase and proteins relating to ganglioside catabolism were also observed. The upregulation of neuroprotective cytokines and limited expression of pro-inflammatory cytokines is consistent with literature indicating their role in signalling pathways during neurodevelopment. Together, this data shows potential Reelin and GM1 gangliosidase signalling during neurodevelopment, and validates neural differentiation of adipose stem cells as a neurodevelopmental model.

Funding Source: The Schwartz Foundation

T-1121

GENE FATE MAPPING AND SINGLE CELL TRANSCRIPTOMIC ANALYSIS OF MENINGEAL-RESIDENT NEURAL PROGENITORS

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We have found that meninges contain a rare neurogenic cell population that gives rise to cortical neurons early after birth in the murine brain in vivo. Using multiple lineage tracing approaches, we found

that lineage tracing experiments indicated that most of the meninges-derived neurons belonged to the PDGFRß-lineage, while only a small fraction of cortical cells originating from meninges was derived from GLAST+ and Nestin+ lineages generated at E13.5. Since PDGFRß is expressed by both pericytes and a subset of radial glia (RG) cells, we hypothesized that the neurogenic meningeal cells belonged to one of these cell types. Single cell transcriptomic analysis identified a PDGFRß+ meningeal cell population with a RG-like gene expression signature. We found meningeal cells with distinct transcriptome signatures characteristic of (i) neurogenic radial glia-like cells (resembling neural stem cells in the SVZ), (ii) neuronal cells, and (iii) a cell type with an intermediate phenotype, possibly representing radial glia-like meningeal cells differentiating to neuronal cells. Thus, we have identified a pool of embryonically derived radial glia-like cells present in the meninges that migrate and differentiate into functional neurons in the neonatal cerebral cortex.

T-1123

MENINGES ARE A SOURCE OF NEUROGENIC CELLS IN NEONATAL MOUSE BRAIN

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Whether new neurons are added in the postnatal cerebral cortex is still debated. Here, we report that the meninges of perinatal mice contain a population of neurogenic progenitors that migrate to the caudal cortex and differentiate into Satb2+ neurons in cortical layers II-IV. These neurogenic meningeal cells are generated during embryonic development between E13.5 and E16.5. The embryonically derived meningeal progenitors remain largely quiescent, and in the first days after birth, they migrate to the cortex and differentiate to cortical neurons, without further proliferation. The resulting neurons are electrically functional and integrated into local microcircuits. In addiction meningeal cells can be isolated from P0 mouse brain and cultured as neurospheres. We analyzed by global transcriptomics (RNA-seq) the signature of the meningeal derived cultured neurospheres and compared it with the one of the bona fide neural stem cells isolated from the VZ and SVZ zone. We found that meningeal derived neurospheres show neurogenic gene expression similar to VZ and SVZ derived neuroshperes thus further suggesting meningeal cell neurogenic signature. These findings broaden the concept of brain plasticity since they indicate that quiescent embryonically-born neural progenitors reside in meninges and they may contribute to add new functional neurons to the postnatal cortex.

T-1125

HESC-DERIVED STRIATAL CELLS GENERATED USING A SCALABLE 3D HYDROGEL PROMOTE RECOVERY IN A HUNTINGTON'S DISEASE MOUSE MODEL

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Huntington's disease (HD) is an inherited neurological disorder characterized by the progressive degeneration of striatal medium spiny neurons (MSNs), which eventually leads to fatal deficits in movement, cognition, and behavior. One promising approach for treating HD is cell replacement therapy, where lost cells are replaced by striatal MSNs or MSN progenitors derived from human pluripotent stem cells (hPSCs). While remarkable prior work paved the way to generate MSNs from hPSCs for application towards HD treatment, as with many stem cell differentiation processes the current state-of-the-art methods for producing striatal MSNs rely on 2D culture systems that typically include poorly defined components, limit scalability, and have yielded mixed therapeutic outcomes in animal models of HD. To take a next step towards clinical translation, here we develop an approach for the efficient generation of striatal progenitors from human embryonic stem cells (hESCs) within a fully defined and scalable PNIPAAm-PEG 3D hydrogel. Following their efficient derivation, transplantation of 3D-derived striatal progenitors into a transgenic mouse model of HD slowed disease progression, improved motor coordination, and significantly increased lifespan by 41%. Importantly, transplanted cells developed an MSN-like phenotype and formed synaptic connections with host cells. Our results illustrate the potential of scalable 3D biomaterials for generating striatal progenitors for HD cell therapy.

T-1127

ENHANCED CORTICAL ORGANOIDS FOR MODELING HUMAN NEURAL DEVELOPMENT AND IDENTIFYING ANTI-ZIKV DRUGS

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The human cerebral cortex possesses distinct structural and functional features that are not found in lower species traditionally used to model brain development and disease. Considerable attention has been accordingly placed on the development of methods to direct the formation of human brain-like structures termed organoids from pluripotent stem cells. However, many differentiation protocols are inefficient and display marked variability in their ability to recapitulate the three-dimensional architecture and course of neurogenesis in the developing human brain. Here, we report optimized organoid culture methods that efficiently and reliably produce cortical and basal ganglia structures similar to those found in the human fetal brain in vivo. Neurons within the organoids are functional and exhibit network-like activities. We further demonstrate the utility of the organoid system for modeling the teratogenic effects of Zika virus (ZIKV) on the developing human brain and identifying therapeutic compounds that can mitigate its destructive actions.

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NEURAL DISEASE AND DEGENERATION

T-1131

ALL-OPTICAL ELECTROPHYSIOLOGY USED TO PROBE PHARMACOLOGICAL MODULATORS ON INTRINSIC EXCITABILITY IN RAT PRIMARY DRG AND HUMAN IPSC-DERIVED SENSORY NEURONS

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Recent advances in stem cell biology offer great opportunities for in vitro human pain models for drug discovery. induced pluripotent stem (iPS) cells derived from patient material can be differentiated into various neuronal subtypes, including sensory neurons. Human iPS cell-derived sensory neurons (hiPSC-SNs) express human proteins, which solves the long-standing issue of differential drug binding between humans and rodents, but suffers from cell immaturity and a lack of relevant in vivo circuits. Here, we use high-throughput optical methods to explore the electrophysiology and pharmacology of primary adult rat dorsal root ganglion (DRG) and hiPSC-SNs, and compare their complex, functional phenotypes. hiPSC-SNs were differentiated using combined small molecule modulation of SMAD, Wnt, FGF and Notch pathways and co-cultured with mouse cortical glia for 35 days before measurement. We used Q-State's proprietary Optopatch all-optical electrophysiology platform to characterize the effects of acute treatment of 13 pharmacological agents, blinded, on both cell types. Stimulation of action potentials was achieved by the blue light-activated channelrhodopsin, CheRiff. Fluorescent readout of voltage was enabled with red light by the Archaerhodopsin, QuasAr. Our custom Firefly microscope enables recording from ~50 individual cells simultaneously with 1 ms temporal resolution and subcellular spatial resolution over a 4 x 0.5 mm field of view. We measured electrophysiology in 10,657 rat DRG neurons and 19,370 iPSC-SNs. Each cellular model showed different basal properties: rat DRG stained positive for Islet1 sensory marker in 98% of cells and exhibited predominantly single-spiking cells in response to a step stimulus whereas hiPSC-SNs expressed ISLET1 in 28% of cells and fired volleys of action potentials. We detected similar, but distinct changes in intrinsic excitability in the two cell types upon compound addition. Select compounds showed changes in spike width and after-hyperpolarization in subpopulations of neurons highlighting the importance of single cell recordings. The Optopatch platform rapidly and robustly characterizes the electrophysiological response of primary and hiPSC-SNs and provides an

information-rich readout of pharmacological effects on neuronal electrophysiology.

T-1133

CHARACTERIZATION OF HUMAN NEURAL PRECURSOR-LIKE CELLS THAT PROMOTE NEUROLOGIC RECOVERY IN THE JHMV MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic degenerative autoimmune disease of the central nervous system (CNS). It is the most common cause of non-traumatic neurological disability in the US. In MS an immune-mediated attack destroys myelin-producing oligodendrocytes in the CNS, disrupting communication between neurons. This leads to a wide variety of specific pathologies depending on the area of the CNS attacked. Previously, using the JHMV mouse model of multiple sclerosis (MS), we showed that intraspinal transplantation of human embryonic stem cell-derived neural precursor like cells (hNPLCs) resulted in a sustained clinical recovery. The mice showed continued clinical improvement for 3 months and sustained recovery for 6 months (longest duration of experiment) post-transplantation. The spinal cord sections of 21 day post-transplant mice showed increased numbers of CD4(+) FOXP3(+) T regulatory cells (Tregs) within the spinal cords and remyelination of spinal neurons. Because we used immunocompetent mice, the transplanted hNPLCs lasted only 8 days post-transplantation. The cells that elicited clinical improvement have a very specific gene expression profile that is unlike any cell type that has been characterized before. We are investigating the developmental potential of these cells to determine whether they share differentiation characteristics with other neural precursors. We are also focusing on secreted proteins made by these cells that may be responsible for the dramatic transient effects of the transplants. Our long term goal is to identify factors that may have clinical use in MS patients.

Funding Source: CIRM Bridges to Stem Cell Research and Therapy Training Grant Trainee

T-1135

EXTRACELLULAR VESICLES DERIVED FROM HUMAN DENTAL PULP STEM CELLS ACTIVATE MIGRATION AND PHAGOCYTIC ACTIVITY OF HUMAN MICROGLIAL CELLS

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Increasing evidence suggests that neuroinflammation has a causal role in the pathogenesis of chronic neurodegenerative diseases. In the present study we investigated the effects of EVs derived from human dental pulp stem cells (DPSCs) on migration and phagocytic activity of human microglial cells. To determine phagocytic activity of immortalized human microglial cells (purchased from ABM) we used apoptotic bodies (AB) derived from ReNcell VM human neural stem cells (Millipore). ABs were prepared and labeled according to the protocol of Dereck, Cronk et al. 2012, with some modifications. The microglial polarization into M1 and M2 states was induced according to the protocol of Gaikwad and Heneka. EVs were purified by differential ultracentrifugation from DPSCs grown in serum- and xeno- free medium. Control and EV-treated M0, M1 and M2 cells were stained with CellTrace calcein green AM (Thermo Fisher Scientific) and incubated with ABs at a ratio 3:1 for 2 hours. Digital images of randomly selected fields were captured by confocal microscope (Leica SP8) and phagocytic activity calculated according to the following formula: number of microglial cells containing engulfed ABs/total number of counted cells × 100. Internalization of phagocytosed material was verified by using Z stacks acquired through confocal microscopy. For wound-healing assays we used 2 well silicone inserts (Ibidi) and Leica SP8 live cell imaging system. Time-lapse microscopy revealed that EVs significantly promoted migration of unpolarized M0 cells. We also detected increased phagocytic activity of M1 and M2 microglial cells (by 40 % when compared with M0 cells). Importantly, EV treatment increased phagocytic activity of M0 and M2 cells by 46 % and 17 %, respectively. By contrast, EVs did not affect phagocytic activity in M1 cells. Our findings demonstrate that EVs derived from human dental pulp can act as potent immunomodulators of human microglial cells.

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T-1137

NEURONS DERIVED FROM ISOGENIC IPS CELL LINES WITH EDITED APOE GENOTYPES DISPLAY DIFFERENCES IN MITOCHONDRIAL RESPIRATION

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The apolipoprotein E (ApoE) allele type 4 is the most prevalent risk factors known for Alzheimer's disease (AD). There are three ApoE alleles - ApoE2, ApoE3, and ApoE4. ApoE4 is associated with a higher risk for AD, whereas ApoE3, the most common allele, is defined as neutral, and ApoE2 is protective. The use of induced pluripotent stem (iPS) cells to model the effect of the different ApoE alleles can be challenging, as homozygous carriers of the ApoE4 or the ApoE2 allele are quite rare. To investigate the function of the different ApoE genotypes, we have successfully used the CRISPR/Cas9 gene editing system to generate a set of isogenic iPS cell lines with a homozygous ApoE4, ApoE3, and ApoE2 genotype, an ApoE knock-out (KO) line as well as the original iPS cell line with an ApoE3 and an ApoE4 allele. All gene-edited lines had a normal karyotype and the same genomic background as confirmed by STR-analysis. After differentiation to neurons, we confirmed the expression of markers specific for the cortical layers relevant for AD. Since mitochondria are often impaired in neurodegenerative diseases, we analyzed their functionality by a Seahorse XF96 analyzer. The experiment revealed significantly higher basal and maximal oxygen consumption in the ApoE2 lines compared to the ApoE4 lines, whereas the oxygen consumption in the ApoE3 line was in between. This finding is in line with previously reported observations in vivo. Interestingly, the ApoE KO line showed the highest respiration indicating that the presence of ApoE might affect mitochondrial respiration. As such, it highlights its importance as a clinical target. In conclusion, these lines capture a robust mitochondrial phenotype and therefore have the potential to serve as an ex vivo tool for future in-depth pathomechanistic analyses or the development of therapeutic drugs.

Funding Source: EBiSC – European Bank for induced pluripotent Stem Cells; Innovation Fund Denmark, EU FP7

T-1139

ACTIVATION OF PSA TO ENHANCE DEGRADATION OF AGGERGATE PRONE PROTEINS

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Pathological aggregation of specific proteins underlies many neurological disorders including Tauopathies, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, and spinocerebellar ataxia. These aggregates often result from the abnormal accumulation of misfolded proteins and impairment of protein degradation pathways. Importantly, reduction of aggregate prone proteins has been shown to be a safe and effective mechanism to slow neurodegeneration in various animal models of these diseases. Recent research has identified the highly conserved puromycin-sensitive aminopeptidase (PSA; NPEPPS) as a potential target in the treatment of Tauopathies. Overexpression of PSA reduces total tau levels both in vitro and in vivo, while PSA overexpression does not cause abnormalities in either mouse or fly models. Interestingly, PSA may also play a neuroprotective role through the direct degradation or regulation of degradation of other aggregate prone proteins that underlie various other neurodegenerative disorders. It has been shown that the inhibition of PSA leads to increased aggregation of mutant and wild type ataxin-3, mutant huntingtin, mutant SOD1, and mutant -synuclein, which are the major components of the aggregates found in spinocerebellar ataxia type-3, Huntington's disease, amyotrophic lateral sclerosis, and Parkinson's disease, respectively. This suggests that PSA may be a key therapeutic target for a number of neurological diseases characterized by protein aggregation, and that activation of PSA may aid in the clearance of accumulated proteins which have the potential to aggregate, leading to a less aggressive progression of the disease in question. A high throughput screen was previously performed and identified compounds which enhanced PSA's proteolytic activity at non-toxic levels. In this study, we test the effects of several of these compounds on the relative concentrations of endogenous forms of these aggregate prone proteins at non-toxic levels in HEK293T cells and primary neuronal cultures.

T-1141

UTILIZING IPSC-DERIVED NEURONS TO ESTABLISH A PRE-CLINICAL RESEARCH PIPELINE FOR RARE NEURODEVELOPMENTAL DISORDERS

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Progress in the development of new therapeutics for neurodevelopmental disorders has been slow, in part, due to a lack of a validated and reproducible human model system with which to model these complex diseases. Here, we show how we have utilized patient-specific iPSC-derived neurons to model cellular phenotypes that may be linked to deletions in chromosome 16p13.11. Heterozygous deletions in this region have been associated clinically with epilepsy, microcephaly, developmental delay, and schizophrenia. We have recently reported a patient with a rare 15-131 kb microdeletion on chromosome 16p13.11. The patient presented with developmental delay and developed psychosis around the age of 6. To model this disorder, we isolated fibroblasts from the patient and an unaffected parent, reprogrammed fibroblasts into iPSCs, and differentiated the iPSCs into cortical and GABAergic neurons. The neurons were analyzed in a series of phenotypic assays to understand the cell and molecular consequences of the deletion, and preliminary results suggest there are increases in soma size and neurite outgrowth in GABAergic neurons from the patient compared with control using morphological analyses on the ThermoFisher Arrayscan XTI. We have also found disruption of mTOR signaling using western blotting, as well as changes in expression levels for genes involved in cell adhesion and G-protein coupled receptor activity identified with whole transcriptome gene expression analysis using the ThermoFisher Ion AmpliSeq system. The generalizability of any phenotypic differences identified in these patient-derived neurons will be confirmed in neurons derived from additional patients with variable 16p13.11 deletions. Future experiments will also focus on utilizing CRISPR to knock out individual genes potentially affected by the deletions in this region to understand the role of each gene in disease phenotype progression. Together, these studies will help identify molecular pathways and cellular phenotypes underlying psychosis in patients with 16p13.11 deletions while establishing a pipeline for identifying robust phenotypic assays for drug repurposing screens to identify novel therapeutic targets for these rare neurodevelopmental disorders.

Funding Source: The Tommy Fuss Center for Neuropsychiatric Research

T-1143

A KNOCK-IN REPORTER ALLOWS CHARACTERIZATION OF TRANSCRIPTIONAL AND EPIGENETIC TRAJECTORIES DURING DEVELOPMENT OF MIDBRAIN DOPAMINERGIC NEURONS

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Parkinson's disease (PD) is the second most common neurodegenerative disease and affects the health of approximately seven million people globally. It is caused by depletion of (mDA) neurons. Generation of mDA neurons from human pluripotent stem cells provides a valuable platform for inquiry into basic and translational studies of PD. However, heterogeneity in differentiation makes it difficult to identify and characterize mDA neurons in culture or in vivo following transplantation. To solve this, we generate a human embryonic stem cell (hESC) line carrying a knock-in tyrosine hydroxylase (TH) reporter. TH is the first and rate-limiting enzyme for dopamine synthesis and is a widely used marker for mDA neurons. We generate the reporter line by integrating RFP (red fluorescent protein) cassette directly downstream of the endogenous TH coding sequence. Our reporter is superior than conventional TH reporters that were constructed on a viral expression vector carrying only the promoter fragments of the gene. In contrast, our reporter is positioned in the same unique chromatin and DNA context as endogenous TH gene, which engages all components of the endogenous TH gene regulation. We validate that our RFP reporter is able to faithfully mimic TH expression during in vitro differentiation and in vivo transplantation. Most importantly, our TH-RFP reporter cell line has enabled, for the first time, purification of mDA neurons from heterogeneous cultures and subsequent characterization. Here, we describe the transcriptional and epigenetic trajectories of mDA neurons differentiated from hESC. Specifically, we provide the first transcriptional profile and global binding maps of H3K27ac, H3K4me1 and 5 hydroxymethylcytosine (5hmC) for purified mDA neurons at four different stages during development. We identified specific enhancers and 5hmC regions for mDA neurons, which are associated to genes critical for cell identity. Based on these data, we predict 78 transcription factors that might play essential roles in mDA neurons development. We anticipate that the tools and data described here will contribute to development of mDA neurons for applications in disease modeling and/or drug screening and cell replacement therapies for PD.

T-1145

PERICYTES EXTEND SURVIVAL OF ALS SOD1 MICE AND INDUCE EXPRESSION OF ANTIOXIDANT ENZYMES IN THE MURINE MODEL AND IN IPSCS DERIVED MOTOR NEURONS FROM AN ALS PATIENT

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Amyotrophic Lateral Sclerosis (ALS) is the most common form of motor neuron disease in adults causing a progressive and irreversible degeneration. Cell therapy is amongst the therapies being tested for this disease. In the central nervous system, pericytes act in formation and maintenance of the blood-brain barrier, a natural defense that slows the progression of symptoms in neurodegenerative diseases. It is well known that in ALS patients, approximately 50% pericytes from the spinal cord barrier are lost. Here we evaluated for the first time the effect of human pericytes in vivo in the SOD1 mice model and in vitro in human ALS motor neurons. For comparison purposes, mesenchymal stromal cells (MSCs) from the same adipose sample were used. Treatment with pericytes extended significantly animals' survival in SOD1 males. Gene expression analysis in brain and spinal cord of end-stage animals showed that treatment with pericytes can stimulate the host antioxidant system. Additionally, pericytes induced the expression of SOD1 and CAT in ALS FUS patient derived motor neurons. Our results suggest that ALS patients may benefit from pericyte transplantation.

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T-1147

MESENCHYMAL STEM CELLS INHIBIT TRANSMISSION OF AMYLOID BETA THROUGH LIPID RAFT-MEDIATED ENDOCYTOSIS

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A hallmark pathological feature of Alzheimer's disease (AD) is the accumulation of extracellular plaques composed of the amyloid-beta (Aβ peptide). Aβ is propagated by cell-to-cell transmission and they affect the onset and progression of AD. To block Aβ transfer is important therapeutic strategies against AD. Using Aβ enriched models, we found that mesenchymal stem cells (MSCs) contribute to the reduction of Aβ endocytosis. And we found that MSCs inhibit Aβ transmission by blocking the lipid raft-mediated endocytosis of extracellular Aβ via modulation of the interaction with lipid raft associated proteins, which led to a prosurvival effect on neurons with functional improvement of synaptic damage and cognitive decline in Aβ-enriched models. The present data indicated that MSCs exert neuroprotective properties through inhibition of extracellular Aβ transmission, suggesting that the property of MSCs may act as a disease-modifying therapy in AD.

T-1149

ER STRESS RESPONSE AND CALCIUM HOMEOSTASIS IS ALTERED IN HUMAN IPSC-DERIVED NEURONS CARRYING THE LRRK2 G2019S MUTATION

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The Leucine-Rich Repeat Kinase (LRRK2) G2019S gain of function gene mutation is one of the most prevalent mutations contributing to Parkinson's disease (PD) pathogenesis. The increased kinase activity alters mitochondrial health, axon outgrowth, intracellular trafficking and autophagy. We have previously shown that human LRRK2 G2019S iPS-derived neurons exhibit increased vulnerability to PD-associated cell stressors and modify mitochondrial dynamics, which can be rescued by LRRK2 inhibitors (Cooper et al., 2012, Sci Transl Med. 2012, 4;4(141):141ra90.). Human iPS-derived neurons carrying the LRRK2 G2019S mutation and challenged with the endoplasmic reticulum (ER) calcium (Ca2+) uptake blocker thapsigargin (THP) show a significant decrease in their ER stress responses accompanied by neurite collapse, when compared to

neurons derived from healthy subject controls. As THP blocks ER Ca2+ influx via sarco/endoplasmic reticulum Ca2+ -ATPase (SERCA) and induces ER stress, this result indicates that iPS neurons carrying the LRRK2 G2019S mutation have an altered capacity to regulate Ca2+ homeostasis. Indeed, we further discovered that after THP-induced SERCA block, human iPS-derived neurons carrying the LRRK2 G2019S mutation exhibit an increase in depolarization-induced calcium influx and modified calcium decay (interpreted as buffering capacity), when compared to healthy subject control neurons. This phenotype is diminished by treatment with antisense oligonucleotides targeting the LRRK2 G2019S mutation. These data suggest that the LRRK2 G2019S mutation alters intracellular calcium homeostasis and ER stress response, potentially contributing to the PD-specific neuronal dysfunction.

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T-1151

BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IMPROVE COGNITIVE IMPAIRMENT IN ALZHIMER'S MOUSE MODEL BY INTRACEREBROVENTRICULAR INJECTION

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Although Alzheimer's disease (AD) is most common cause of dementia, effective treatments have not been fully developed. Here we aimed to investigate whether bone marrow derived mesenchymal stem cells (BM-MSC) can ameliorate cognitive impairment in mouse model of AD via intracerebroventricular (icv) injection. Female B6C3-Tg mice (APPswe, PSEN1dE9, 85Dbo/J: APP/PS1) which develop Aβ plaques in the brain and exhibit cognitive decline were used. BM-MSC were isolated from SD rats and 1.0×105 of BM-MSC was administered icv in APP/PS1 mice at age 13 months old, 2 times with 2-week intervals. At 2 weeks after last injection, cognitive function was evaluated using Morris Water Maze (MWM) test and mice were sacrificed for morphological study. We also injected PKH-labeled BM-MSC icv to evaluate the distribution of BM-MSC in the brain. In MWM test, cognitive impairment in APP/PS1 mice was completely recovered by BM-MSC treatment. In the subiculum region of the hippocampus, the positive area of Aβ plaques in APP/PS1 mice injected with vehicle (APP/PS1+V) was significantly increased compared to wild type mice (WT), and this increase was not altered in APP/PS1 mice injected with BM-MSC (APP/PS1+BM-MSC). The density of NeuN-positive neurons in APP/PS1+V was significantly decreased than that in WT, and this decrease was not altered in APP/PS1+BM-MSC. On the other hand, TNF-α expression in GFAP-positive astrocytes was significantly increased in APP/PS1+V, and this increase was suppressed in APP/PS1+BM-MSC. In electron microscopy, degradation of postsynaptic density (PSD) as well as decrease in synaptic number was found in APP/PS1+V, and these abnormalities were reversed in APP/PS1+BM-MSC. After icv injection of PKH-labeled BM-MSCs, labeled cells were found in lateral ventricle attached to the choroidal plexus. The results suggest that icv injected BM-MSC contributes to repair the damaged astrocytes in AD mice and increase the synaptogenesis, where exosomes secreted from BM-MSC might have neuroprotective roles by internalization into astrocytes and neurons.

T-1153

GROWTH DIFFERENTIATION FACTOR 11 (GDF11) IMPROVES CNS FUNCTION IN AGED MICE

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Aging represents one of the biggest risk factors for and the most common component of neurodegenerative diseases. Parabiosis experiments, whereby young and aged mice are surgically connected to share a common circulatory system, have demonstrated that the brain functionality in aged mice can be improved by exposure to young blood. Multiple factors, some of which act positively, some negatively, are likely to be involved. Our lab showed that Growth Differentiation Factor 11 (GDF11), a member of the Transforming Growth Factor beta (TGFβ) superfamily of proteins that is present in the blood of young and old mice, is one of the positive factors and can improve the vasculature and number of neural stem cells in the subventricular zone (SVZ) of aged mice when administered systemically. Our new experiments have demonstrated that GDF11 administration also increases neurogenesis in the subgranular zone (SGZ) of the dentate gyrus and improves vasculature in the hippocampus. Notably, GDF11 administration also increases DeltaFosB immunoreactivity in the hippocampus of aged mice, suggesting that it enhances neuronal activity as well. Although GDF11's mechanism of action is unknown, it is highly plausible that GDF11 does not cross the blood brain barrier (BBB). It likely exerts its effects by acting on brain vasculature, which consequently enhances neurogenesis and improves neural function. Whether ameliorating the aging process will slow down disease progression or even promote functional recovery in the degenerated brain is unknown. However, restoring function to a brain whose neurons and vasculature are

already damaged would have tremendous therapeutic potential in the neurodegeneration field.

T-1155

BIOACTIVITY AND MICRO RNA PROFILE OF MESENCHYMAL STEM CELL NEURAL PROGENITOR-DERIVED EXTRACELLULAR VESICLES

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Mesenchymal stem cell-derived neural progenitors (MSC-NPs) are currently being investigated clinically as a novel cellular therapy aimed at promoting repair and regeneration in patients with progressive multiple sclerosis (MS). Recent results from an open-label phase I clinical trial demonstrated functional neurological improvement in 15 out of 20 progressive MS patients after repeated intrathecal MSC-NP injection. Pre-clinical studies in the EAE mouse model of MS showed that neurological recovery after MSC-NP injection was associated with increased spinal cord myelination, decreased immune infiltration in the CNS, and increased recruitment of endogenous progenitor cells. In addition, in vitro co-culture experiments demonstrated trophic and immunomodulatory effects of MSC-NP cells or conditioned media. The specific mechanism by which MSC-NPs exert proximal influence on CNS progenitor populations remains unknown. Extracellular vesicles (EVs) consisting of exosomes and/or microvesicles mediate cell-to-cell communication through transfer of protein and miRNA cargo. We investigated whether MSC-NP-derived EVs could mediate some of the reparative effects of these cells in models of MS. MSC and MSC-NP-derived EVs were purified from 3 day cell-conditioned media by membrane affinity and size exclusion columns. EV concentration and size were quantitated by tunable resistive pulse sensing technology (qNano). Purified EVs were added to cultured rat neural stem cells on day 0 of differentiation by growth factor withdrawal. EVs were found to exert a trophic effect on oligodendroglial differentiation resulting in an increase in the number of mature galactosylceramidase-positive oligodendrocytes. In order to identify candidate micro RNAs (miRNAs) that mediate the trophic effects of MSC-NP EVs, we analyzed EV miRNA expression by qPCR and demonstrated expression of a panel of miRNAs including miR-let7c and let7f. Additional results from miRNA profiling from EVs will be presented. These results suggest that the trophic effects of MSCs and MSC-NPs in MS can be mediated through EV release.

CANCERS

T-1159

YIN YANG 1 ORCHESTRATES A METABOLIC PROGRAM THAT IS REQUIRED FOR NEURAL CREST HOMEOSTASIS AND MELANOMA FORMATION

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There is increasing evidence that cancer cells highjack developmental programs to mediate disease initiation and progression. Melanoma is a neural crest-related cancer that originates from atypical melanocytic behavior, and is known to be a highly heterogeneous and invasive cancer type. These properties are reminiscent of the broad differentiation potential and high migratory capacity of neural crest (NC) cells. The aim of this study was to identify new transcription factors that are required for NC development and to determine whether these factors also play a role in melanoma disease initiation and progression. To address this question we have performed a microarray analysis comparing migratory neural crest stem cells (NCSCs) to NC cells primed to differentiation. By using an integrated system for motif activity response analysis (ISMARA) we observed that NCSCs displayed higher Yy1 motif activity than their primed counterparts. Yy1 is a transcription factor known to play pleiotropic roles, namely in proliferation, differentiation and survival in a cell type dependent manner. To dissect the in vivo role of Yy1 during NC cell development, we conditionally ablated Yy1 in the pre-migratory NC. Strikingly, loss of Yy1 in the NC resulted in agenesis or hypoplasia of various NC derivatives. Further, later ablation of Yy1 in post-migratory NC resulted in no overt phenotype. We next sought to determine whether Yy1 plays a role in melanoma initiation or progression. To do so, we conditionally ablated one copy of Yy1 in the melanocytic lineage of the TyR:: N-Ras Q61K Ink4a-/- melanoma mouse model. By combining RNA-Seq, ChIP-Seq and untargeted metabolomics we observed that Yy1 controls a set of genes involved in distinct metabolic pathways and that Yy1 inactivation leads to drastic changes in metabolism. Thus, Yy1 appears to regulate a metabolic program associated with NCSC homeostasis and tumorigenesis.

T-1161

ELEVATED FOXG1 AND SOX2 IN GLIOBLASTOMA ENFORCES NEURAL STEM CELL IDENTITY THROUGH TRANSCRIPTIONAL CONTROL OF CELL CYCLE AND EPIGENETIC REGULATORS

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Glioblastomas (GBM) are heterogeneous aggressive brain tumours driven by neural stem cell-like cells. GBM stem cells frequently express high levels of the transcription factors FOXG1 and SOX2. Here we show that increased abundance of these regulators restricts NS cell differentiation and can trigger de-differentiation of post-mitotic astrocytes. Shared transcriptional targets identified include cell cycle (e.g. Foxo3, Plk1, Nmyc) and epigenetic regulators (e.g. Dnmt1, Tet3, Chd3). Foxo3 is confirmed as a critical downstream effector. Foxo3 loss combined with exposure of cells to the DNA methylation inhibitor 5-Azacytidine is sufficient to enforce de-differentiation, while ablation of the conserved FOXG1/SOX2-bound cis-regulatory repressive element in Foxo3 restricts FOXG1/SOX2-induced de-differentiation. Patient-derived GBM stem cells in which FOXG1 is genetically ablated display reduced proliferation, increased differentiation, and upregulation of FOXO3 protein in vivo. These findings suggest elevated FOXG1/SOX2 in GBM drives unconstrained proliferation and restricts terminal differentiation by transcriptional control of both core cell cycle regulators and epigenetic targets.

Funding Source: SP is supported by a Cancer Research UK Senior Research Fellowship

T-1163

FORCED EXPRESSION OF REPROGRAMMING FACTORS (OKSM) IN HUMAN MELANOMA CELLS ALTERS CANCER-RELATED PATHWAYS AND TUMORIGENICITY

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Cancer cells can acquire pluripotent character by forced expression of reprogramming factors, leading to the deletion of epigenetic memory and resulting in decreased tumorigenicity. However, studies on cancer cell reprogramming are limited and contradictory. In this study, the main aim is to investigate the effect of cellular reprogramming on the tumorigenicity of cancer cells. MDA-MB-435, G361 human melanoma cells and human primary melanocytes (as a positive control) were transfected with Sendai viral vectors encoding the OKSM factors. After 3 weeks of culturing, colonies have emerged in the reprogrammed melanocytes whereas cluster of cells with distinctive morphology were observed in the transfected cancer cells. OKSM factors were upregulated in both cancer cells and primary melanocytes. All pluripotency markers showed increased expression in the reprogrammed melanocytes; however only Nanog expression was observed in the transfected cancer cell lines. When miRNA expression profiles were compared with microarrays, a significant difference was observed between naive and transfected melanoma cells. Gene targeting and pathway analysis showed that various cancer related pathways including p38/MAPK got activated. Embryoid body formation assay used to evaluate the pluripotent character of the transfected cells. Reprogrammed melanocytes showed expression of all lineage markers. Transfected MDA-MB-435 overexpressed FGF-5 and downregulated Brachyury; whereas G361 overexpressed both FGF-5 and Brachyury, compared to naive cancer cells; indicating that there were changes in epithelial to mesenchymal transitions. In order to understand the tumorigenicity of these resulting cells, sensitivity of chemotherapeutic agents, response to oxidative stress and invasion potential were compared. MDA-MB-435 cells were shown to decrease tumorigenicity after transfection with OKSM factors; whereas G361 increased tumorigenicity. These results suggest that even though transfection of cancer cells with OKSM factors were not able to fully reprogram cells towards pluripotency, it changed the tumorigenicity and modified cancer related pathways. Therefore cancer cell reprogramming could be a promising strategy to further understand cancer biology and develop novel methods for therapy.

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T-1165

IMAGE ANALYSIS OF CELLULAR EPIGENETIC LANDSCAPES IDENTIFIES A MULTIPARAMETRIC SIGNATURE OF GLIOBLASTOMA DIFFERENTIATION

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The resistance of high-grade glioma to conventional cytotoxic drugs has prompted the development of novel approaches to therapy, including differentiating glioma stem cells (GSCs) to less tumorigenic cell fates. However, the comprehensive transcriptomic profiling required to identify differentiated GSCs is not readily applicable to high-throughput drug screening, particularly at the level of single cells. To determine the differentiation state of GSCs we analyzed the topography of the epigenome of single cells using automated microscopy and machine learning. "Microscopic Imaging of Cellular Epigenetic Landscapes" (MICEL) captures patterns of nuclear staining for epigenetic marks from which derived texture features are used to compare different populations of cells to identify a classifier that accurately distinguishes them. MICEL enabled discrimination between cell types with high accuracy and derivation of image-based signatures of drug-induced perturbations. An epigenetic signature characteristic of differentiated glioblastoma cells was identified and validated for high-throughput drug screening to identify compounds that differentiate GSCs into non-tumorigenic cells.

T-1167

INVESTIGATING THE RATES OF ASYMMETRIC CELL DIVISION IN MEDULLOBLASTOMA CANCER STEM CELLS

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Medulloblastoma is a very aggressive brain tumor common in children up to age four. The available therapies are not effective for most of medulloblastoma patients, and many efforts have been done to understand this tumor behavior – including tumor etiology, progression and genetic and cellular heterogeneity. The presence of cancer stem cells (CSC) correlates with medulloblastoma aggressiveness and might be important to tumor initiation and progression. One important feature of stem cells is the capability to undergo asymmetric cell division (ACD) – a mitosis that ensures the formation of one stem cell and one cell committed to differentiation. CSC are also able to undergo ACD, but these cells display mechanisms to favor symmetric divisions, generating high proliferative cells that contribute to tumor growth. The present work aims to investigate ACD in medulloblastoma cells and define how these divisions occur and are regulated. Three medulloblastoma cells lines (DAOY, D283 and P13-USP-Med) were cultured as tumorspheres (TS) for 7 days, when the CSC population was enriched (CD133, Nestin and Sox2 positive cells). Cell differentiation and ACD rates were studied after TS dissociation followed by cell culture in different culture medium (DMEM-F12 supplemented with N2+B27, N2+B27+EGF+FGF or 1% FBS) for 2 to 7 days. For the first time ACD was described in medulloblastoma cells. Cells cultured in the presence of N2+B27 showed increased ACD rates than cells cultured in presence of FBS. This was observed in all cell lines. The addition of EGF and FGF decreased the rates of ACD in a cell line dependent way. Increased rates of ACD correlated with increased levels of neuronal differentiation, indicating that ACD could favor cell differentiation. These results show that ACD rates can be modulated in medulloblastoma CSC and allow us to regulate ACD to study its relation to medulloblastoma aggressiveness.

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T-1169

SMAD SIGNALLING PROMOTES MELANOMA METASTASIS INDEPENDENTLY OF PHENOTYPE SWITCHING

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Malignant progression of melanoma is thought to require the dynamic shifting of neoplastic cells between proliferative and invasive phenotypes. Contrary to this conventional "phenotype switching" model, we now show that augmented SMAD-dependent signaling in melanoma leads to emergence of malignant cells co-expressing the phenotype switching markers MITF and AXL and displaying both proliferative and invasive properties. Specifically, conditional deletion of Smad4, which abrogates canonical SMAD signaling, prevented tumor initiation and proliferation in vivo, pointing to the requirement of a pro-proliferative ligand for melanoma formation. Ligand screening revealed that BMP7 promotes melanoma cell proliferation and overrides the effects of pro-invasive TGF-β2 or NODAL. However, in cells concomitantly exposed to pro-proliferative and pro-invasive ligands, inactivation of the inhibitory SMAD factor SMAD7 induced the expression of pro-invasive transcription factors in proliferating melanoma cells. Accordingly, conditional Smad7 deletion in vivo resulted in massive metastasis formation. Thus, modulation of SMAD-dependent signaling can contribute to malignant transformation of the disease by overcoming phenotype switching.

T-1171

COPAIBA OIL AFFECTS THE VIABILITY OF CELL LINES (FIBROBLAST AND LEUKEMIA) AND STEM CELLS

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Oil extracted from the tree trunks of the Copaiba genus is widely used in folkloric medicine for a variety of pathologies. This study has evaluated the components in a copaiba oleoresin formulation by gas chromatography (CG) and their effect on cell viability for 24h of cultivation in three types of human cell cultures: pulp of deciduous exfoliated teeth (SHED), promyelocytic leukemia cell line (HL60) and lung fibroblast cell line (MRC5) by MTT assay. GC-MS evaluation indicated a prevalence of the sesquiterpenes beta-caryophyllene (41.9%), alpha-trans-bergamotene (17.3%) and beta-bisabolene (12.1%) in the copaiba oil. Three groups of different concentrations of copaiba oil were used: G1: zero (control), G2: 125, G3: 300 and G4: 500 µg/mL. In the SHED, G2 promoted cell viability of 103.2±13.9% (mean ± standard deviation) without statistic difference from the control (100.0±11.5%). G3 and G4 promoted significantly lower cell viability (84.0±14.8%, $p \leq 0.01$ and 7.2±5.2%, $p \leq 0.01$) than the control. The results of viability on the cell lines (fibroblast and leukemia) showed that the oil promoted a decrease in cell viability with statistic differences in all doses compared with the control ($p \leq 0.01$). The viability of the HL60 cells was, respectively, 100.1±8.8%, 78.8±11.3, 50.8±15.9 and 32.1±28.2% for G1 to G4. The viability of MRC5 was 100.0±9.8%; 88.3±13.1%; 59.9±12.8% and 5.0±2.3% for G1 to G4, respectively. In conclusion, low concentrations of copaiba oil were not cytotoxic to the SHED; however, the use of copaiba oil decreased SHED viability in high concentrations. All concentrations of copaiba oil promoted a decrease in cell viability in the MRC5 and HL60 cell lines. Although copaiba oleoresin is widely used in traditional medicine, caution must be taken for medical application due to its cytotoxicity; however, the oil components should be studied for their possible anti-tumor potential.

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T-1173

ISOLATION AND CHARACTERISATION OF STEM CELLS FROM ORAL CANCER CELL LINES

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Cancer stem cells (CSCs) are a subset of the tumour cell population which possess the capability for indefinite self-renewal and differentiation to diverse heterogeneous cell lineages that form the bulk of the tumour. Moreover, they are highly tumorigenic, play a crucial role in invasion and metastasis and also have the ability to resist anticancer therapy. Identifying and isolating CSCs is crucial to study their characteristics with the aim of subsequently targeting and eradicating them. CSC isolation has previously been mainly based on the expression of specific markers or clonogenicity as well as the rapid adherence of such cells to extracellular matrix proteins. We hypothesize that CSCs can be isolated from oral cancer cell lines using cell adhesion assays. The aim of this study was to develop adhesion assays to isolate cells from OSCC cell lines and investigate the stem cell characteristics of these cells, compared to unsorted cells. Early adherent cells (EACs) were isolated from 2 different OSCC cell lines (H357 and SCC4) on the basis of their rapid adherence (within 10 minutes) to fibronectin (FN). The stem cell characteristics of these EACs were investigated using of colony forming and proliferation assays and FACs analysis. The growth rate of EACs of both cell lines was two fold lower and the colony forming efficiency three fold higher than unsorted cells ($p < 0.05$). FACs analysis of H357 cells showed that early adherent cells expressed increased levels of CD44 ($p < 0.001$), while early adherent SCC4 cells expressed increased levels of CD29 compared to unsorted cells ($p < 0.001$). The results of this study show that rapid adherence to FN effectively isolates a sub-population of cells from cell lines which show stem cell like characteristics of growth and colony forming but only some cell surface stem cell marker expression. Further work will study the stability of this population.

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CHROMATIN AND EPIGENETICS

T-1175

GLOBAL REORGANISATION OF CIS-REGULATORY INTERACTIONS UPON LINEAGE COMMITMENT OF HUMAN EMBRYONIC STEM CELLS

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Long-range cis-regulatory elements such as enhancers coordinate cell-specific transcriptional programmes by engaging in DNA looping interactions with target promoters. Deciphering the interplay between the promoter connectivity and activity of cis-regulatory elements during lineage commitment is crucial for understanding developmental transcriptional control. Over the last decade, chromosome conformation capture and derived methods have enabled the biochemical mapping of looping interactions to offer new insights into their architecture across different cell types. In particular, Hi-C has allowed genome-wide characterisation of higher-order chromatin dynamics during cell differentiation at the level of contact domains and topologically associated domains. However, despite these advances, the global and unbiased high-resolution mapping of promoter cis-regulatory interactions that form and remodel during development and stem cell differentiation is still lacking. We recently developed Promoter Capture Hi-C (PCHi-C) that uses sequence capture to enrich Hi-C libraries for interactions involving the promoters of most annotated genes. Here, we use PCHi-C to generate a high-resolution atlas of chromosomal interactions involving ~22,000 gene promoters in human pluripotent and lineage-committed cells. Our data links thousands of known and predicted enhancer elements with their putative target genes, including those known to drive tissue-restricted reporter gene expression in transgene assays. We reveal extensive dynamics of cis-regulatory contacts upon lineage commitment, including the acquisition and loss of promoter interactions. This spatial rewiring occurs preferentially with predicted changes in the activity of cis-regulatory elements, and is associated with changes in target gene expression. Our results provide a global and integrated view of promoter interactome dynamics during lineage commitment of pluripotent cells, and offer new insights into the cis-regulatory logic underlying the transcriptional control of early human development.

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T-1177

CHROMATIN ALTERS THE LINEAGE POTENTIAL OF MOUSE EMBRYONIC STEM CELLS

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Embryonic stem cells (ESCs) are pluripotent cells that can form all cell types of the developing embryo. During early embryonic development, they lose their pluripotency status and differentiate into more specialized cells. This differentiation process is guided through a gene expression program that is regulated precisely and in a timely manner. Elucidating the exact differentiation mechanism for ESCs is important for both basic and medical sciences. ESC differentiation bears significant reverse similarity to reprogramming of mature cell types (induced pluripotent stem (iPS) cell formation). Many factors that play a role in ESC differentiation were also found to be involved in the iPS formation. Additionally, tumorigenesis and ESC differentiation show mechanistic parallels. Therefore, understanding how ESCs form mature cell types might hold critical value in both reprogramming and cancer research. ESC lineage commitment into mesoderm, endoderm and neuroectoderm is one of the earliest cell fate decisions in development. We investigated the role of epigenetic factors in coordinating the accurate and timely gene expression program required for ESC commitment into mesodermal and endodermal fate. We identified Arid4b protein as an essential factor in both mesoderm and endoderm lineage commitment. However, Arid4b loss did not affect neuroectoderm differentiation. Arid4b is in the Sin3a corepressor complex. Our data shows a physical interaction specifically with histone deacetylase Hdac1, but not with Hdac2. Transcriptomic analysis of differentiating ESCs showed a lack of expression in lineage specific pioneer transcription factors in arid4b or hdac1 knockout cells. We are currently investigating the detailed epigenetic contributions of Arid4b in differentiating ESCs.

T-1179

CONCURRENT EPIGENETIC REPROGRAMMING OF THE 3D CHROMATIN LANDSCAPE IN GROUND STATE PLURIPOTENCY

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Embryonic stem cells (ESCs) cultured in serum-supplemented or defined 2i-medium (with two kinase inhibitors) represent two distinct states of pluripotency that display different transcriptional and epigenetic landscapes. Here, we performed a systematic and integrative analysis of the transcriptional dynamics, transcription factor (TFs) modules, enhancer activation and 3D chromatin organization during the conversion between serum- and 2i-ESCs. We show that the early phase of reprogramming to 2i-ESC is characterized by altered expression of TFs and chromatin accessibility. H3K27-acetylation of enhancers occurs at later stages and we show that it is functionally linked to nuclear receptor family. Capture HiC shows that the extensive transcriptome and epigenome reprogramming takes place with only minor promoter-enhancer rewiring. Instead concurrent epigenetic marking of enhancers embedded in highly interconnected chromatin interaction communities correlates with differential gene expression. Our study provides conceptual insights into the role of chromatin community in dynamic epigenetic marking and gene regulation in ESCs.

T-1181

ZBTB2 CAN REGULATE GENE EXPRESSION FROM UNMETHYLATED CPG ISLAND PROMOTERS

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DNA methylation plays a key role in cellular differentiation through regulation of gene expression. Proteins that bind to the DNA depending on its methylation status, so-called readers of either unmethylated or methylated DNA, are thought to play an important role in DNA methylation-mediated gene expression. We used quantitative proteomics to identify such readers and study their dynamics during differentiation. Using DNA pull-downs followed by mass spectrometry, we found a module of three zinc finger proteins that have a strong preference for unmethylated DNA, both in undifferentiated mouse embryonic stem cells (ESCs) and in more differentiated neural progenitor cells

(NPCs). We then used chromatin immunoprecipitation and deep sequencing to show that ZBTB2, the most abundant of these zinc fingers, binds at CpG island promoters in mESCs in vivo. Binding intensity at these sites decreases in NPCs, likely reflecting the global increase in DNA methylation during differentiation. Integrating these data with whole transcriptome analysis, we see that that ZBTB2 binding is enriched at active genes, which is confirmed by the fact that upon knockout of this protein in mESCs, more genes go down in expression than go up. Moreover, a subset of ZBTB2-bound genes seem to be directly controlled by ZBTB2, implying a role in gene regulation. Knockout of Zbtb2 leads to an upregulation of TET1 and TET2, which is accompanied by a significant increase in global 5hmC levels. In addition, the expression of pluripotency factors such as ESRRB, NANOG and KLF4 changes upon Zbtb2 knockout, suggesting that the pluripotency network of these cells is disturbed. We propose that ZBTB2 is a DNA methylation sensitive transcription factor that is involved in cellular differentiation.

ORGANOIDS

T-1183

LGR5-POSITIVE SUPPORTING CELLS IN THE COCHLEA: REPLACEMENT OF HAIR CELLS IN RESPONSE TO WNT

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Death of cochlear hair cells, which do not regenerate, is a cause of hearing loss in a high percentage of the population. Routes to regeneration of hair cells would provide a treatment for hearing loss. Wnt signaling is required for the differentiation of hair cells during embryogenesis. Wnt stimulates otic progenitors to express transcription factor, Atoh1, which is required for hair cell development. Activation of Wnt signaling facilitates regeneration of hair cells in newborn cochlea in vitro and in vivo. Lgr5, a downstream target of the Wnt pathway, and a protein that marks intestinal epithelial stem cells, is expressed in a subset of supporting cells in the sensory epithelium of the cochlea and these cells are hair cell progenitors based on lineage tracing. A combination of drugs to stimulate Wnt signaling and inhibit histone deacetylase expanded these cells as organoids, significantly increasing the small number of progenitors obtained from the cochlea. The cells could be converted to hair cells in high yield. Lgr5-positive cells had distinct phenotypes from the other (Sox2-positive) supporting cells and differentiated to hair cells at a higher rate, consistent with these cells playing a role as hair cell progenitors. Lgr5-negative cells did not differentiate to hair cells. Hair cell replacement was seen in a spontaneous response to damage following hair cell death in neonatal ears and was due to supporting cell transdifferentiation to hair cells, directly, or after cell division. The response to damage was accompanied by Wnt release and was blocked by inhibition of Wnt signaling. Supporting cells in neonatal ears were responsive to hair cell death demonstrated by an upregulated Lgr5 and increased canonical Wnt signaling activities. Based on lineage tracing, upregulation of Wnt signaling in the newborn inner ear, even in the absence of damage, specifically targeted the Lgr5-expressing cells, leading to proliferation and transdifferentiation to hair cells. Our findings highlight the potential of Lgr5-positive supporting cells in the cochlea as a regenerative pool to new hair cells. Pharmacological stimulation of Wnt signaling pathways may provide new therapeutic routes to hair cell regeneration.

T-1185

IN VIVO ENGRAFTMENT OF MULTICELLULAR LUNG ORGANOID INTO PULMONARY NICHE

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Translating advances in lung stem cell biology into functional tissue repair remains challenging. One critical limitation is the lack of available methods to generate ex vivo, and transplant in vivo, complex three-dimensional lung tissues suitable for eventual translational applications. Embryonic lung development led us to propose that three-dimensional lung-bud formation can be recapitulated in vitro by co-culturing lung epithelial cells with endothelial and mesenchymal lineage cells. Previously we have demonstrated that multicellular airway organoids derived from adult human airway cells are able to self-organize and develop into lung tissue-like structures with well-organized microvascular and luminal structures, tissue-like segregation of mesenchyme and epithelium, and both proximal and distal epithelial differentiation (Biomaterials, 2017). We now provide evidence that these organoids are mechanically stable and can be handled for transplantation directly into pulmonary niches at either pleural or bronchial locations, establishing proof of concept for lung organoid transplantation. To advance toward clinical translation, we also now generate organoids formed from lung progenitors derived from human induced pluripotent stem cells following established protocols. Lung organoids derived from adult human airway cells or human lung progenitors derived from GFP+ iPSCs were successfully implanted into the thoracic cavity of immunocrompromised mice

on the pleural or bronchial surface via fibrin glue gel attachment plus VEGF (100ng/ml). Organoid capacity for in vivo growth, differentiation, integration and maturation over 4 weeks in both thoracic implantation models is currently under evaluation. At week 1, we have positively identified engrafted organoids using Cell-Tracker labelling, human specific antibody staining and/or GFP+ signals. Organoid epithelial cells display positive staining for the proliferation marker ki67 and epithelial marker CDH1, alveolar type 2 cell marker SPC and alveolar type 1 cell marker AQP5, consistent with distal lung epithelial differentiation in vivo. Taken together these results support the potential for long term engraftment of lung organoids in the pulmonary niche.

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T-1187

COMPARATIVE ANALYSIS OF MOUSE AND HUMAN KIDNEY DEVELOPMENT TO INFORM IN VITRO NEPHROGENISIS

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In development of novel curative options for kidney diseases, knowledge gained from studying murine kidney development has informed the derivation of kidney cell types and organoids from mouse and human pluripotent stem cells. However, to move these in vitro derived organoids toward translational applications, it is essential to appreciate human-specific nephrogenic programs. Indeed, initial characterization in the McMahon laboratory has revealed some striking differences across species, questioning the assumption that human and mouse share progenitor programs. My colleagues in the McMahon lab and I have performed extensive characterization of human nephrogenesis and created a bench mark to assess current differentiation protocol. We examined whether kidney organoid formation undergoes processes similar to normal human kidney development. We also utilized human ESC reporter lines which enable isolation and characterization of in vitro derived kidney cell types. Our analyses suggest that while kidney organoids contain kidney-like cell types, their formation does not fully recapitulate human kidney development. More importantly, the analyses allow us to propose strategies to improve in vitro nephrogenesis.

Funding Source: California Institute for Regenerative Medicine; National Institute of Diabetes and Digestive and Kidney Diseases; (Re)Building a Kidney

T-1189

ARYL HYDROCARBON RECEPTOR REGULATES SELF-RENEWAL CAPACITY AND TUMOR EVOLUTION FROM GLIOMA STEM-LIKE CELLS IN PATIENT-DERIVED TUMOR ORGANOIDS

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The mechanisms that maintain self-renewal capacity in glioblastoma stem-like cells (GSC) are still unknown. Here we show that Aryl hydrocarbon receptor (AhR) and AhR target genes are functionally expressed on GSCs. Activating AhR is sufficient to increase the self-renewal capacity of CSC, the canonical gene expression of CYP1A1 and other AhR targets on GSCs. In contrast, inhibition of AhR leads to a decrease of self-renewal capacity and AhR targets genes. We showed that putative AhR genes increase during GSC differentiation and can be targeted at both the GSCs and differentiated stages of tumor progression in vitro. Using a novel "reporter" organoid model of glioblastoma from GSCs carrying OLIG2-GFP-Luc dual transcriptional reporters, we demonstrated that AhR inhibition decreases proliferation, increased apoptosis, reduce the size of the organoids and the expression of OLIG2. Furthermore, increasing the apoptotic niches in the organoids. Using a complementary approach, we demonstrated that AhR knockdown in GSCs induced loss of self-renewal with increased in p53 phosphorylation. Implantation of AhR deficient human GSCs leads to a decrease in tumor size and survival advantage in mice. Furthermore, using microarray gene expression analysis of patient-derived organoids, we established the molecular programs affected by targeting AhR in these organoids, corresponded to genes related to stemness and inflammation that are independently associated with survival. Our findings established the functional expression of AhR in GSC as critical to maintaining tumor growth and self-renewal in vivo and in a patient-derived

model of glioblastoma during two critical stages of tumorogenesis. Our results indicate that tumor derived-organoids can be used as a preclinical predictive model to study the molecular vulnerabilities of tumors to distinct candidate therapies in a given patient, toward personalized Neuro-Oncology.

Funding Source: NRI Career Development Funds

T-1191

PROBING HUMAN-SPECIFIC CORTICAL EXPANSION USING STEM CELLS-DERIVED CORTICAL ORGANOIDS

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How the evolutionarily enlarged and folded human neocortex is developmentally regulated remains obscure, mostly because of the lack of appropriate system approaching human development. Lately, human pluripotent stem cell derived organoids revolutionize the study of human development and related disorders, promising a novel platform to decipher the species-specific features of human brain development. By combining CRISPR/Cas9 based gene-editing in human embryonic stem cells (hESC) with a streamlined protocol of human cerebral organoids, we established a system in which all genetic modified hESC lines can be generated from a master line of hESC bearing a set of tet-on inducible Cas9 in its AAVS1 locus, and organoids aggregated from hESCs are cultivated in suspension using an optimized recipe. Using this system, we can efficiently generate the desired genetic modified hESC lines for cerebral organoid production and maintain organoids for up to 12 months without compromising their viability and quality. These cortex organoids sequentially stratified into layers of cells that express specific markers of the cortical layers, such as PAX6, SOX2 and TBR2 of VZ/SVZ zone, and TBR1, CTIP2, SATB2, BRN2, CUX1, REELIN of the six cortical layers, respectively. In particular, specialized out radial glia (oRGs) in outer subventricular zone (oSVZ) labeled with HOPX and FAM107A can also be identified in these cortical organoids. To demonstrate the liability of this system, we employed a human specific mirco-RNA, and found this miR sustains the neural progenitor pool in germinal zones through targeting neuronal genes such as NeuroD2 and Emx2, which implied a possible model of human cortex expansion. We thus established a reliable system to study human brain development, which is particularly valuable to unravel the evolutionary uniqueness that contribute to enlarged brains in hominoids.

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T-1193

THREE-DIMENSIONAL EVALUATION OF HUMAN EMBRYONIC STEM CELL-DERIVED CEREBRAL ORGANOID

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The mammalian cerebral cortex has a multilayered complex structure, and an expansion of cortical area in human is thought to be the key factor of human specific higher brain functions such as language, cognition, and so on. Recent advances in pluripotent stem cell technology have enabled to generate 3-dimensional (3D) human organoid model in various tissues including cerebral cortex. Using SFEBq (serum-free floating culture of embryoid body like aggregates with quick reaggregation) method, we have investigated several neural models such as cerebral cortex, medial pallium, and choroid plexus. There are several important aspects in human cerebral organoid to address human developmental processes, for instance, formation of layered structure and folding of cerebral tissues. Thus, one direction to progress these works is the analysis of these tissues in 3D-order. However, we currently lack the means to evaluate cerebral organoids in 3D-order. Here, using a rarefaction technology, we demonstrate the 3D-evaluation of cerebral organoid. First, we developed a human embryonic stem cell culture method to induce cerebral organoid that contained layered cortical tissues as in vivo. Following long-term culture, these cerebral cortical tissues gave rise to cortical projection neurons and layered structure gradually thickened. Then, we utilized X-Clarity system for rarefaction tool. Combined with 3D-imaging, we have successfully examined the structure of whole cerebral organoid. By easy accessibility to the structure of cerebral organoid, our approach will have potential to address future themes for human developmental process or disease modeling.

T-1195

TET1-MEDIATED DNA HYDROXYMETHYLATION IS ESSENTIAL FOR ADULT LIVER DUCTAL PROLIFERATION

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The adult liver exhibits low physiological turnover. However upon damage it activates a proliferative response that results in the regeneration of the tissue upon injury. The molecular mechanisms behind this phenomenon are largely unknown. We recently described that adult ductal liver cells can be isolated and expanded as liver organoids from healthy mouse and human liver tissue. Liver organoid cultures express ductal (e.g. EpCAM), and stem cell markers (e.g. Lgr5) whilst retaining their physiological features, as they are capable to differentiate into functional hepatocyte both in vitro and in vivo after transplantation. We found that the vast majority of liver organoid-forming cells are arrested in G1 and that entry into S-phase takes ~40hrs after isolation, thus suggesting that liver organoid culture mimics in vitro the activation of the proliferative response of the ductal compartment in vivo, upon damage. By analysing gene expression profiles of ductal cells in organoid culture conditions, we found that several epigenetic regulators are dynamically regulated during organoid formation. Specifically, genetic depletion of Tet1, belonging to the family of proteins responsible for oxidizing 5-methylcytosine (MC) to 5-hydroxymethylcytosine (hMC), impaired organoid formation. Moreover, decreased Tet1 levels impaired Lgr5 expression and maintenance of organoid cultures while ectopic expression of Tet1 (but not its catalytically inactive form) rescued this phenotype, thus indicating that Tet1-dependent hMC is required for initiation and maintenance of adult liver ductal progenitors in vitro. At the molecular level, Tet1 and hMC levels increase at the transcriptional start site (TSS) of Lgr5 prior to the acquisition of the stem cell signature, thus suggesting that Tet1 regulates the expression of stem cell genes in adult liver organoids. Importantly, following liver injury in vivo, Tet1 hypormophic mice exhibited significantly reduced numbers of proliferating ductal cells and decreased Lgr5 expression, which affected proper regeneration of the ductal compartment. In summary, we found that the epigenetic factor Tet1 plays a crucial role in the activation of the stem-cell signature in adult liver ductal progenitors both in organoid culture and in vivo, upon tissue injury.

TISSUE ENGINEERING

T-1197

HIGH-CONTENT ASSAYS FOR MORPHOLOGICAL CHARACTERIZATION OF NEURONAL DEVELOPMENT IN 3D MATRIX USING HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONAL CULTURES

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There is an increasing interest in using three-dimensional (3D) cultures for assay development and translational biology. 3D cultures have the advantage of closely recapitulating aspects of the human tissues including the architecture, cell organization, cell-cell and cell-matrix interactions, and more physiologically relevant diffusion characteristics. Hydrogels are widely used as an artificial extracellular matrix to grow neural cells in a 3D environment. The fully synthetic hydrogels were developed pre-casted in a 96-well plate featuring an in-depth surface density gradient promoting the infiltration in 3D of cells deposited on the hydrogel surface (3DProSeedTM hydrogels). The focus of the present study was to develop a high- throughput 3D neurite outgrowth assay using iPSC-derived neurons, with the long term goal of establishing more predictive 3D models for neurodegenerative and neurotoxicology screens. Human iPSC-derived neurons were plated into 3D hydrogel matrix and demonstrated development of complex networks in 3D volume. Development of networks was monitored in transmitted light, or after staining with Calcein AM or fluorescent-conjugated TuJ1-antibodies. High-content imaging, including confocal acquisition and 3D analysis, were used for evaluation of neuronal networks. We optimized cell culture, staining in a 96-well assay format, and also developed imaging and 3D analysis protocols for assessing morphological phenotypes and viability of neurons in 3D matrix. Phenotypic readouts included quantitative characterization of the extent and complexity of neuronal networks by multiplexed read-outs including measuring neurite outgrowth, number of processes and branches, as well as cell number and viability. For 3D analysis we have developed a custom module measuring numbers and volumes of neurons (fibers), as well as number of branching points and cell viability. We characterized multiple quantitative read-outs, and tested a series of gold-standard-compounds with known neurotoxic properties. We observed concentration–response effects of selected compounds

and illustrated how the proposed method can be used for high-throughput compound toxicity screening and safety evaluation.

T-1199

PORCINE BLASTOMERES CONTRIBUTE TO INTRASPECIES CHIMERA IN A LMX1A/PITX3 KNOCKOUT BLASTOCYST COMPLEMENTATION SYSTEM

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Blastocyst complementation is a developing field which has the potential to grow authentic human tissue within genetically engineered livestock, thereby providing an alternative source of human tissue suitable for clinical transplantation. We believe that this technology can be harnessed to obtain genuine human midbrain dopamine (mDA) progenitors for transplantation in Parkinson's disease. Our previous study demonstrated that human stem cells were unable to contribute to the developing porcine fetus following microinjection into PITX3 knockout porcine embryos. We hypothesized that the human stem cells were unable to respond to the porcine microenvironment and were outcompeted by host tissue. Furthermore we hypothesized that the niche created by knockout of the late-onset of PITX3 expression is too late in development for human cells to occupy. To address these hypotheses, we complemented LMX1A/PITX3 knockout porcine embryos with GFP+ porcine blastomeres and extracted fetuses at embryonic day 30. LMX1A is a transcription factor involved in mDA specification and is expressed earlier in development in comparison to PITX3 while porcine blastomeres are better suited to contribute to the porcine embryo. Immunohistochemical analysis demonstrate that GFP+ cells contributed to the complemented fetus, with GFP+ cells observed throughout the brain and spinal cord. Investigation of the ventral mesencephalon revealed immature and mature dopaminergic cells with fibers extending in the medial forebrain bundle. Through confocal fluorescence microscopy GFP+ cells were observed not to co-localize with dopaminergic precursors. The majority of GFP+ cells in chimeric fetuses have radial glia morphology with fibers running perpendicular to ventricular spaces. In line with our previous results, this study suggest that donor cell compatibility is a crucial factor for blastocyst complementation. As no complemented cells were observed in the ventral mesencephalon, it is hypothesized that greater manipulation of the niche will be necessary for successful complementation of neural tissue. Future studies will utilize transgenic mice in an attempt to identify new protocols for generating mDA progenitors through blastocyst complementation.

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T-1201

3D CARDIOMYOGENIC MICROTISSUES FOR IN VITRO ASSAYS AND HEART REPAIR

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The use of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) has advanced cardiac research. But, in contrast to native tissue, most of the in vitro models used to date have been largely two dimensional. Recent studies have indicated that 3D models might be more physiologically relevant and should therefore aid in creating a more accurate representation of in vivo conditions. Therefore, the goal of this study is to establish and characterise 3D cardiomyogenic microtissues, either homotypic (consisting of hPSC-CMs only) or heterotypic (consisting of hPSC-CMs combined with other relevant cell types such as fibroblasts and/or endothelial cells (ECs)). Both types of the aforementioned microtissues have been generated in 96U-well low attachment or microcavity array plates. Homotypic microtissues consisted of hESC-CMs, differentiated using a previously established protocol (with a purity of >96% based on flow cytometry for selected cardiac markers), while heterotypic microtissues consisted of these CMs, combined with human fibroblasts (HFFs) and/or umbilical cord-derived ECs. Final optimal cell ratios (based on systematic testing as well as other studies) were CMs only, CMs:HFFs 70:30, CMs:ECs 70:30, CMs:ECs:HFFs 70:15:15 and CMs:ECs:HFFs 33:24:43. Furthermore, testing of different media resulted in an optimized medium for all three cell types. In all cases the microtissues formed within ~24h and started to contract within 3-5 days. The inclusion of fibroblasts seemed to aid in the formation and structure of the microtissues, while the inclusion of ECs seemed to aid in the contractions of the microtissues (creating pronounced contractions at higher frequency). Finally, extracellular flux analysis of

these 3D microtissues, using the Seahorse XF system, has been optimized to enable bioenergetics/metabolic measurements in living tissues. Future work will focus on further characterization of morphological and physiological aspects of these microtissues as well as comparisons to native cardiac tissue, in order to elucidate the interaction of the various cell types found in the heart and provide a more functionally relevant in vitro model.

T-1203

SCALABLE BIOREACTOR EXPANSION OF HUMAN PLURIPOTENT STEM CELLS AND DIFFERENTIATION TO PANCREATIC PROGENY

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Diabetes mellitus refers to a group of chronic metabolic disorders characterized by hyperglycemia due to autoimmune destruction of insulin-producing β-cells (type 1; T1D) or to extensive β-cell exhaustion and depletion often exacerbated by insulin resistance (type 2; T2D). Whole pancreas or pancreatic islet transplantation has the potential to restore normoglycemia, but this approach is severely hampered by the shortage of donor tissue and the life-long immunosuppression required post-transplantation. Human pluripotent stem cells (hPCSs) can serve as a renewable source of cells because of their ability to expand extensively and to be differentiated into various cell types. However, their production in clinically relevant quantities will be necessary. Unlike static cultures utilized in current protocols for pancreatic differentiation, bioreactors are an attractive modality for scalable expansion and directed differentiation of hPSCs to islet cells in part because of their homogeneous environment. Moreover, automated bioreactors would enable the monitoring and control of critical culture variables such as pO2, pH, and factor concentration, ensuring consistency in cell production. In spite of that, efforts for the adaptation of pancreatogenic differentiation methods to stirred-suspension cultivation of hPSCs have been limited. We have been successful in propagating hPSCs as aggregates and on microcarriers in stirred-suspension, achieving higher densities than in static cultures with cell viability over 80% and limited cell lysis (LDH activity). Subsequent to expansion, cells are subjected to directed differentiation toward endoderm with over 80% expressing SOX17 and FOXA2. Ongoing work focuses on further coaxing hPSC-derived definitive endoderm cells toward PDX1-positive cells. Our work will contribute to the development of technologies enabling the robust scalable production of therapeutics for diabetes.

T-1205

DEVELOPMENT OF A HUMAN CARDIAC ORGANOID INJURY MODEL REVEALS INNATE REGENERATIVE POTENTIAL

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The adult human heart possesses a limited regenerative potential following an ischemic event, and undergoes a number of pathological changes in response to injury. While cardiac regeneration has been documented in zebrafish and neonatal mouse hearts, it is currently unknown whether the immature human heart is capable of undergoing complete regeneration. Combined progress in pluripotent stem cell differentiation and tissue engineering has facilitated the development of human cardiac organoids (hCO), which resemble fetal heart tissue and can be used to address this important knowledge gap. This study aimed to characterise the regenerative capacity of immature human heart tissue in response to injury. Following cryoinjury with a dry ice probe, hCO exhibited an endogenous regenerative response with full functional recovery by two weeks following acute injury. Cardiac functional recovery occurred in the absence of pathological fibrosis or cardiomyocyte hypertrophy. Consistent with regenerative organisms and neonatal human hearts, there was a high basal level of cardiomyocyte proliferation. Following cell cycle inhibition with mitomycin C treatment, functional recovery was not seen at 14 days post cryoinjury. This study suggests that immature human heart tissue has an intrinsic capacity to regenerate.

T-1207

DEVELOPMENT OF HEPATIC BLOCKS USING HUMAN ADIPOSE TISSUE-DERIVED STEM CELLS THROUGH THREE-DIMENSIONAL CELL PRINTING TECHNIQUES

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Currently, most acute liver diseases are treated through liver transplantation. However, transplantation is

limited by organ donor scarcity and immune rejection response. Moreover, only three types of commercial bio-artificial livers are available, and these have marginal effects on survival rates. Therefore, tissue regenerative medicine using stem cells has been developed to solve such unmet medical needs. In this study, we attempted to differentiate human adipose stem cells (hASCs) into hepatocyte-like cells (AHLCs) and fabricate liver regenerative hepatic block scaffolds, consisting of only hASCs and AHLCs, through three-dimensional cell printing using a neutralized type I atelo collagen solution derived from porcine skin. Hepatic blocks were also treated with genipin, a natural cross-linking reagent, in order to maintain the structure and strength of collagen scaffolds, which contained hASCs and AHLCs. The hepatic blocks were then transplanted into the livers of SD rats to confirm safety and efficiency. The results showed that hASCs and AHLCs inside the hepatic blocks translocated into the portal vein of hepatic lobules in SD rats by 4 weeks after transplantation, as demonstrated by immunohistochemical staining using human nuclear-specific antibodies. Moreover, serum biochemistry, which was altered after induction of acute liver failure by dimethylnitrosamine, returned to normal in hASC scaffold-transplanted rats, and increased levels of interleukin-10 expression were observed in the livers of these rats. Thus, these hepatic blocks consisting of hASCs could be used as alternative bio-artificial livers to facilitate the regeneration of damaged liver tissue.

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T-1209

DEVELOPING 3D NANOFIBER SCAFFOLD AND MICROFLUIDIC CHAMBER TO EFFICIENTLY MODEL THE ALZHEIMER'S DISEASE PATHOGENESIS

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Increasing evidence indicated that three dimensional (3D) cell culture systems are more advantageous, comparing with 2D cultures, in terms of mimicking the in vivo environment with high physiological relevance. The combination of these 3D culturing models and the stem cell technologies hold promise for studying the pathogenesis and therapeutic strategies of complex human neurological diseases, such as Alzheimer's disease (AD). Here, we developed a novel 3D culturing system, combining 3D nanofiber scaffold and microfluidic chamber to provide a controllable circulation system for the 3D cultured cells, to better understand the key events in AD pathogenesis, including amyloid-β (Aβ) accumulation and tau hyperphosphorylation. To achieve this, we seed the glutamatergic and cholinergic neurons, which derived from human AD patient iPS cells, on the 3D nanofiber scaffold. We optimized various parameters of the nanofiber scaffold fabrication, including the polymer materials, scaffold pore size, degradation time of the scaffold, and electrical properties of the nanofiber, to manage to promote iPSCs differentiation towards neurons and promote the long term survival of iPSC-derived neurons. We found that iPSC-derived neurons from AD patients are more robust in modeling AD disease when they are cultured on 3D nanofiber scaffold comparing with cultured in 2D system. Furthermore, by controlling the culture circulation conditions via microfluidic chamber, we found the microenvironment can significantly affect the development of AD pathology. Taken together, these preliminary results indicated that this novel controllable 3D culture system which combines 3D nanofiber scaffold and microfluidic chamber is more advantageous comparing with current 2D and 3D cell culture system to modeling AD disease by using iPSCs-derived neurons from AD patients.

T-1211

CRISPR CORRECTION OF PATHOLOGICAL HOMOZYGOUS LOW-DENSITY LIPOPROTEIN RECEPTOR MUTATION IN FAMILIAL HYPERCHOLESTEROLEMIA INDUCED PLURIPOTENT STEM CELLS

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Familial Hypercholesterolemia (FH) is a genetic deficiency of the low-density lipoprotein receptor (LDLR) that results in hypercholesterolemia and accelerated development of cardiovascular disease (CVD). Receptor defective homozygous FH (HoFH) patient's LDL-C can approach 1000mg/dl. In this project, we hypothesized that genetic correction of LDLR mutations will restore normal cholesterol metabolism in receptor defective HoFH. We obtained HoFH fibroblasts from Coriell Cell repository (GM03040) and confirmed the deficiency in receptor mediated endocytosis of fluorescently labelled LDL (DiI-LDL). The LDLR was sequenced (Ambry Genetics) and a homozygous 3bp deletion in exon 4, chromosome 19 was identified (c.654_656delTGG) and confirmed in house by Sanger sequencing. This mutation has been identified as FH-Piscataway, a class II mutation. We confirmed the lack of mature protein (160kDa) even in the presence of lovastatin by Western blot. The

fibroblasts were DNA fingerprinted and karyotyped (Cell Line Genetics) before (and after) reprogramming with modified mRNA. Colonies were live labelled with Tra-1-81-488 and positive colonies selected for subculture. The HoFH-iPSC expressed typical pluripotent proteins and differentiated to hepatocyte-like cells. To target the 3bp deletion for CRISPR/Cas9 modification, we used the Feng lab's MIT CRISPR Design Tool to identify potential single guide sequences (sgRNA) and appropriate guide pairs for use with Cas9 nickase (Cas9n). We obtained Cas9, Cas9n and sgRNA plasmids from Addgene (# 60599, 60600 & 60601) deposited by the Hotta lab and cloned in our selected sgRNA. We designed a single strand oligodeoxynucleotide (ssODN) repair template to insert the missing 3bp, introduced a novel XmnI restriction site and silent mutations to minimize guide and Cas9 rebinding after genome editing. HoFH-iPSC were electroporated with a NEPA21 using combinations of Cas9/Cas9n, sgRNA and ssODN. Post transfection, cells were selected with Hygromycin for 5d then expanded in culture. To determine LDLR activity, transfected cells were treated with lovastatin overnight and exposed to DiO-LDL for 4h. Within each culture were DiO deficient and competent colonies, indicating successful correction and restoration of LDLR mediated LDL-C endocytosis.

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T-1213

COMPREHENSIVE GENE EXPRESSION ANALYSIS OF HUMAN MESENCHYMAL STEM CELLS CULTURED ON THE MICRO ELASTICALLY-STRIPED PATTERN GEL MATRIX

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Recently it was revealed that the mechanical field of the culture environment of human mesenchymal stem cells (hMSC) contributes to the determination of differentiation lineage. We are developing a culture substrate that controls the differentiation direction or the maintaining undifferentiated state of hMSC, by approaching to oscillate the input of mechanosignals received from stiff and soft regions in the process of cell migration. In this study, the micro elastically-striped pattern gel matrix was prepared and the gene expression pattern of hMSC cultured on the gel was characterized. The micro elastically-striped pattern gel matrices were prepared by the following method; a sol solution containing 30 wt% aqueous solution of photo-cross-linkable gelatin with sulfonyl camphorquinone was put between glasses, then a stiff / soft region was fabricated by irradiating a bright / dark stripe pattern of 50 μm / 50 μm using a reduced projection maskless photolithography. hMSCs were cultured on the soft gel (< 10kPa), the stiff gel (70-100kPa) or the soft and stiff striped pattern gel (PG), respectively, for 7 days, then DNA microarray experiments and pathway analyses were performed. By culturing hMSC on the soft gel, the gene expressions of AKR1C3 which controls cell proliferation and / or differentiation, RGCC involved in cell cycle, EGR1 related to proliferation, apoptosis and differentiation, EGR2 involved in cell migration, and IGFBP1 promoting cell migration were significantly increased. SPP1 and EGR1 involved in bone differentiation and NPTX1 and NMB related to neuronal differentiation were also increased. By culturing hMSC on PG, FOXQ1, GREM1, ANXA10 involved in cell cycle, differentiation and proliferation, CD36, ANK3, ARHGAP22, FGD4 involved in cell adhesion / movement / skeleton, CALB2, AK5, NMB involved in cranial nervous system, and SPP1 were significantly increased, and WNK1, PTPRR involved in cell proliferation / differentiation, INA related to cranial nervous system, PRLR, GRIN2A, ADRA2C, PANX2, NPTXR, GRM6, LAMA3, AHNAK related to cell migration were induced. The pathway analyses showed that "size of body", "homing of cells", "differentiation of cells", "cell movement", and other functions related to cell size, differentiation and migration predicted to be increased by culturing hMSC on PG.

T-1215

ELECTROSPUN POLYURETHANE-GELATIN SCAFFOLDS FOR MANUFACTURING SKIN SUBSTITUTE

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There is an immediate need for skin substitute despite significant developments in the management of severe skin loss. Gelatin is a low cost, natural biomaterial which is frequently used for tissue engineering applications and serve as a potential home for progenitor cells. However it suffers from a lack of sufficient mechanical strength and associated difficulties with handling. Polycarbonate urethanes (PU) are biodegradable elastomeric biomaterials with water and CO2 as final degradation products. These polymers can be spun into fibrous scaffolds, with excellent cell compatibility, controlled

degradation, and non-toxic degradation products. We hypothesized that the addition of a small amount of PU to gelatin would improve the mechanical strength of electrospun gelatin. A new gelatin-based electrospun scaffold was fabricated for skin tissue engineering via the addition of PU. Screening different ratios of Gel and PU, we found that scaffolds generated with a ratio of gelatin to PU of 80:20 by mass (Gel80-PU20) exhibited no significant difference in average fiber size and fiber morphology, however the yield strength, and elongation of these scaffolds increased relative to 100% gelatin scaffolds (Gel100). These properties are essential for the optimal performance of the scaffold in vivo. Human dermal fibroblasts (HDF) were employed as one of the main cell sources in the dermis. More than 90% of the cells were viable, comparable to the Gel100 in an in vitro assay. Unlike the HDF cultured on Gel100 scaffolds which showed an aligned orientation, HDF cultured on Gel80-PU20 had a random orientation, reminiscence of human skin. The depth of cell infiltration into the scaffold was similar for Gel100 and Gel80-PU20, as well as for commercial skin substitute material IntegraTM. The results show that Gel80-PU20 scaffold is an ideal 3D environment for essential cell component of skin and might serve as an ideal scaffold for manufacturing skin substitute using various skin progenitor cells.

Funding Source: Toronto Hydro, Canadian Institutes of Health, CFI Leader's Opportunity Fund

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

T-1217

ORGANOID TECHNOLOGY: IS COMMERCIALIZATION MORALLY ACCEPTABLE

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Organoid technology makes it possible to grow human tissue in vitro. The technology promises various scientific and translational applications, and commercial interest is rapidly growing. An increasing number of patents is issued, and organoids can be commercially banked, distributed, and used. Organoids can be of particular interest for the pharmaceutical and biotechnological sector, as they can innovate the drug development pipeline. If organoids proof to be safely and effectively transplantable in patients, they can enter the market as therapeutic products. Commercial parties can contribute to bringing biomedical products from the bench to the market. Nonetheless, commercialization of bodily material is known to raise ethical concerns related to justice and the commodification of the human body. Empirical research on the attitude of tissue donors and the general public echoes the sensitivity of commercial involvement. We therefore ask: Is commercialization of organoid technology morally acceptable? This question can traditionally be answered with two opposite responses: the gift or market paradigm. The gift paradigm entails that human bodily material may never give rise to commercial gain, and that exchange and use takes place in the public domain. The market paradigm embraces that bodily material should be regarded as a commodity that can be commercially exchanged and used. We contend that organoids cannot be captured in either gift or market paradigm. The paradigms are underpinned by a problematic divide between persons and things. Persons are normally said to have personal rights and they cannot be owned nor sold. Things can be subject to property rights, and they can be sold if they have exchange value. The gift paradigm conceptualizes human tissue as 'persons', whereas the market paradigm frames tissues as 'things'. Organoids, however, are neither person nor thing. They are hybrids that ambiguously relate to persons, bodies, technologies, and markets. Therefore, commercialization of organoids is possible, and can be morally acceptable under certain circumstances. A middle way should be sought that balances the relation of organoids to persons and their bodies, with the need for commercial involvement to translate the scientific merits of organoids from the bench to clinical care and society.

T-1219

DECISION-MAKING IN TRANSLATION: PATIENT AND PHYSICIAN VIEWS ABOUT OFF-LABEL HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MULTIPLE SCLEROSIS

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The development of health technologies traditionally follows a linear path from discovery through clinical research and market approval. In the stem cell arena, however, the path incorporates different points of access to interventions outside of clinical trials. We examine the case of multiple sclerosis (MS) for which, following significant clinical research, patients may be faced with decisions about off-label stem cell transplants. We explore the decision-making analyses of both MS patients who received an off-

label hematopoietic stem cell transplant (HSCT) and clinicians responsible for their care. We conducted a series of semi-structured interviews with 10 MS patients and 13 MS specialists. Audio-recorded interviews were transcribed verbatim and analysed for recurring themes and individual variations using the constant comparative approach. Patients described a sense of urgency to access HSCT, given a limited therapeutic window and rapidly progressing disease. While clinicians explained that HSCT is not a first line therapy given its risk profile, patients rationalized the risks of the procedure, including the risk of mortality, with its potential therapeutic benefits and fears associated with the natural course of the disease. Patients articulated altruistic motivations for receipt of HSCT. On the other hand, clinicians were ambivalent about the societal benefit of off-label routes of HSCT, citing concerns that off-label administration may result in translational delays and reduced uptake by the medical community compared with interventions that proceed to the clinic through traditional clinical trials. The data reveal lingering tensions about balancing opportunities for access and evaluation in the context of off-label HSCT application for MS while promoting common good. Articulation of both converging and diverging patient and clinician concerns and priorities about HSCT for MS will promote shared decision-making and informed hope in clinical care.

Funding Source: Stem Cell Network Canada (Impact Research Agreement FY17/PP2, JI), Canadian Institutes of Health Research (INV-126709 AT), University of British Columbia Faculty of Medicine (SB).

T-1221

OUTREACH ACTIVITIES TO HIGH SCHOOL STUDENTS ON THE ETHICAL AND SOCIAL ISSUES ASSOCIATED WITH STEM CELL RESEARCH AND REGENERERATIVE MEDICINE IN JAPAN

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The Japanese government has pushed forward with stem cell research (SCR) as a significant part of clinical research. Eventually, regenerative medicine (RM) would become one of the most preferred treatment options in the future. The "Pharmaceutical, Medical Device, and Other Therapeutic Products Act" was enacted in Japan in November 2013 and implemented in November 2014. In most of the cases, ordinary medicine is the one documented with proven effect and efficacy. However, because of the characteristic of cellular products, this act enables an expedites the approval system by allowing stem cell product to be released to the market after securing not only the efficacy but the safety of the product. This means that efficacy and safety will be further confirmed after many patients have the cellular products implanted. This is a radical change from the previous traditional drug development policies. It is surmised that many Japanese people still do not recognize this change. There would still be people who would neither be able to tell what stem cells are nor would they understand the risks and benefits associated with SCR and therapies. It is in this context that our team believes that our public outreach to educate people on SCR and RM is so significant. Thus, we began our public outreach by visiting a high school to give lectures and provide discussions on the topic of the ethical and social issues relevant to potential achievements that can be gained from SCR. We selected high school students with the age extending from fourteen to sixteen years old. This age is considered as the threshold between both informed assent and consent. What is more relevant is that these adolescents will not have mentors in their family to help them and provide advice regarding the issue because their parents are also the ones who encounter the unfamiliar cell therapy for the first time. We will visit a high school in Kochi, Japan to have a seminar on March 11th, 2017. This seminar will be scheduled for 90 minutes. We will design teaching tools to help students understand what SCR and RM are all about. We will also address what ethical and social issues to consider in using such technologies. Our poster will present more information on the seminar and on the potential challenges associated with this outreach activity.

Funding Source: This research is funded by the Research Center Network for the Realization of Regenerative Medicine associated with Japan's Agency for Medical Research and Development, AMED.

T-1223

GLOBAL TRENDS OF RESEARCH USING INDUCED PLURIPOTENT STEM CELLS

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The induced Pluripotent Stem Cells (iPSCs) has been reported for 10 years or more. iPSCs could be handlable for top-ranked researchers in their early days, but now are available for almost all and applicable for many kinds of researches, meaning that the phase of researches using iPSCs has been progressed. We aimed to investigate the phase transition of research using iPSCs in this research. Methods: "IPSCs" was set as Mesh term. Trends of research using iPS cells from 2006 to recent years were extracted from the PubMed database. From

those papers, only original works have been extracted after exclusion of review, commentary, historical articles nor news. The resultant 3323 papers have been classified into their main themes, reprogramming, differentiation protocols for specific cells and/or tissues, pathophysiological researches of diseases and drug discovery, and then the trends over the years have been elucidated. In the next step, we focused into the fields of pathophysiological researches of diseases and drug discovery, papers, whose number were 590, have been selected by their Impact Factor (IF) (Thomson Reuters) 6 or more, resulted in 232 papers. The IF of each papers have summed up year and year and the mass has been shown as trends of researches. The trend of research activities of reprogramming and differentiation protocols for specific cells and/or tissues have peaked out in 2013 and 2014. On the other hand, the activities of the pathophysiological researches of diseases and drug discovery have increased rapidly from 2013 to now. The 232 papers in these researches areas with 6 or more IF showed that their main target diseased were in the order of nervous system, immune/blood, cardiovascular and digestive organs. Researches using iPSCs have become utilized as a general tool for pathophysiological researches of diseases and drug discovery.

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CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

T-1225

ALLOGENEIC BONE MARROW MESENCHYMAL STROMAL CELL THERAPY IN PATIENTS WITH STEROID-REFRACTORY GVHD

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Background: Graft-versus-host disease is a serious complication following allogeneic stem cell transplantation. Mesenchymal stromal cells (MSCs) have emerged as a promising candidate for treatment of steroid-refractory GVHD. Methods: In this single group, open label, phase I/II interventional study, we report safety and efficacy of third-party bone marrow MSCs administered intravenously to GVHD patients who were resistant to 2 to 4 immunosuppressive drugs, prior to MSC infusion. A panel of 11 analytes including cytokines, growth factors and hormones were tested by Luminex assay before MSC infusion and at the time of initial response measurement to investigate effect of MSC therapy on circulating cytokine levels in GVHD patients. Results: A total of 28 MSC infusions were given to 10 patients suffering from acute (n=6) or chronic (n=4) GVHD. Median MSC dose was 4.8 million cells/Kg (range 0.81-21.2 million). Three patients suffering from advanced stage GHVD died before completion of initial response period and were labeled as non-responders. All remaining seven patients are alive and five of them have sustained complete remission during a median follow up of 12 months and currently are off immunosuppression. The other two patients had good initial response and received repeat MSC course to sustain the response. None of these patients developed any complication or toxicity related to MSC infusion. The overall and disease-free survival was 70% and 50% respectively. ALuminex analysis revealed a modest drop in levels of pro-inflammatory cytokines like IFNγ, TNFα, IL-1β, IL-2, IL-4, IL-6, IL-17A and IL-17F after MSC infusion. Conversely an elevation in serum IL-10 levels was witnessed after MSC therapy. The markers of growth and proliferation found to be increased only in patients showing response to MSC treatment, compared to a slight decline in non-responders who ultimately died of GVHD related complications. Discussion: Third-party donor-derived bone marrow mesenchymal stromal cells therapy is a safe and feasible treatment for steroid-refractory GVHD patients. (NCT02824653)

T-1227

OPTIMIZING THE CORRECTIVE DONOR CONSTRUCT FOR CRISPR/CAS9 MEDIATED SITE-SPECIFIC GENE MODIFICATION OF CD40 LIGAND THROUGH CRISPR-BLOCKING MUTATIONS

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X-Linked Hyper-IgM (X-HIGM) is a primary immunodeficiency characterized by the inability to undergo immunoglobulin class switching in B cells. As a result, minimal levels of IgG, IgA, and IgE are present in X-HIGM patients, which leads to an increased susceptibility to pathogenic infections. A defect in the CD40L gene on the X chromosome is responsible for X-HIGM. It is our goal to use the CRISPR/Cas9 gene editing platform to make a site-specific cut within the endogenous CD40L gene and subsequently utilize homology-directed repair (HDR) to insert a corrective cDNA donor sequence, to wholly restore CD40L function while maintaining its control under the endogenous

promoter. Although we are able to achieve high rates of correction at CD40L (up to 50%), we also detect significant re-cleavage of our inserted donor in both K562 erythroleukemia cells as well as CD34+ peripheral blood stem cells (PBSC's). We hypothesize this occurs because the Protospacer Adjacent Motif (PAM) which the Cas9 uses as one of the recognition patterns to cleave dsDNA still exists in the corrective donor. By changing the S. pyogenes Cas9 PAM sequence within our donor from canonical 5'-NGG-3' to 5'-NHH-3', we could potentially mitigate re-cleavage events of the integrated donor by CRISPR/Cas9. Preliminary data in K562 cells has demonstrated that the incidence of re-cleavage events decreases from 98.4% to 7.0% when the endogenous PAM is altered to NAA. Additional work is being done to investigate other PAM modifications and to determine if gene correction rates are affected by these CRISPR-blocking mutations in the donor.

T-1229

EVADING IMMUNE RECOGNITION AND REJECTION WITHOUT SYSTEMIC IMMUNE SUPPRESSION

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The promise for stem cell-derived cell therapies is enormous. However, patient-specific approaches will not likely be economically realistic in the near future given the time and costs to derive, modify and characterize individual stem cell lines. Practical solutions for many therapies will be those that use clinically-tested allogeneic cells from an HLA-matched source. The biological barrier inherent to this approach, however, is the need for systemic suppressive drugs that prevent immune rejection. Yet these drugs do not always work, and have serious side effects including increased risks of infections and cancer, as well as liver and kidney damage. Our goal is to engineer cells that control their microenvironment and prevent immune rejection in allogeneic settings, while leaving the host's systemic immune system fully competent. We have collected a set of known immunomodulatory genes involved in cancer immune escape, including those found in transmissible cancers of dogs and Tasmanian Devils, the foetus and placenta that prevent rejection by mothers, and parasitic worms. We are combining our solution to prevent rejection of therapeutic allografts with the fail-safe cell system developed by the Nagy lab (see Qin Liang's (Nagy lab) poster), and expect to transform the paradigm for the clinical translation of cell therapies.

Funding Source: This work has been supported by grants from CIHR foundation scheme, Canadian Research Chair, Medicine by Design (University of Toronto), and the Ontario Institute for Regenerative Medicine (University of Toronto).

T-1231

DEPLOYING CELL THERAPY IN THE SUBSTANTIA NIGRA OR NUCLEUS BASALIS OF MEYNERT DURING DEEP BRAIN STIMULATION SURGERY IN PATIENTS WITH PARKINSON'S DISEASE

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We have developed a strategy to address several of the regulatory, practical, and ethical issues that arise from designing clinical trials examining biological therapies requiring surgical delivery. We are in the process of carrying out two Phase I studies with the primary goal of examining the safety and feasibility of autologous peripheral nerve grafts delivered to target brain areas at the time of deep brain stimulation (DBS) surgery in patients with Parkinson's disease. DBS therapy is FDA approved for the treatment of several conditions including Parkinson's disease. We chose peripheral nerve tissue as donor material because Schwann cells, after injury, transdifferentiate to become "repair cells," activate antioxidant response element signaling pathways, and release a host of neurotrophic factors. Our cell therapy delivery strategy has helped us clear many of the hurdles encountered in early-stage clinical trials. Three key advantages of our design are 1) participants have their own sural nerve removed and transplanted at the time of DBS surgery, without any significant modifications, thus the delivery of the grafts does not require FDA oversight; 2) DBS surgery is an insurance reimbursable procedure, thus greatly reducing trial costs, and 3) DBS is a standard of care for Parkinson's disease thus patients do not have to forego the therapeutic benefits of DBS to participate in the trial. We have transplanted grafts into the substantia nigra and/or nucleus basalis of Meynert in 34 participants without any severe adverse events related to the grafting procedure. For the 17 individuals who received a graft to the substantia nigra and had the surgery at least more than a year ago, 11 have shown clinically important improvements in their movements when we tested their underlying responses while temporarily off their therapy. Meanwhile, after almost a year, 16 individuals who received only DBS and not a graft showed no improvement (an almost one point worsening on the Motor Part of the Unified Parkinson's Disease Rating Scale). While dedicated efficacy studies are needed to assess the potential efficacy of this therapy, we are finding that this approach of combining

cell therapy at the time of DBS surgery provides substantial advantages.

Funding Source: Supported by gifts to the Brain Restoration Center; Tom Dupree for Parkinson's Disease Research; Pro's Players Fore Parkinson's; and the National Center for Advancing Translational Sciences, through NIH grant UL1TR000117.

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

T-2001

NANOG BLOCKS PRIMITIVE HEMATOPOIESIS DURING MOUSE GASTRULATION

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As one of the main transcription factors of the gene regulatory network responsible for embryonic pluripotency in mammals, Nanog maintains the undifferentiated state of the inner cell mass and epiblast of the embryo and of embryonic stem (ES) cells. In order to address if Nanog is sufficient to perform this role beyond pre-implantation stages, we have used a TetOn inducible Nanog overexpressing transgenic mouse model and performed transcriptomic analysis at different time windows of Nanog overexpression in the post-implantation embryo. This analysis has allowed us to identify different gene networks affected by Nanog. Surprisingly, a large fraction among the genes downregulated as a consequence of Nanog overexpression was related to hematopoiesis. Therefore we hypothesize that Nanog may play an important role in preventing the expression of the early hematopoietic regulators, as we could confirm by quantitative PCR. These observations may reveal a new role for Nanog in the embryo, suggesting that individual pluripotency factors can be acting in the early regulation of specific lineages in the embryo.

Funding Source: The CNIC is supported by the Spanish Ministry of Economy and Competitiveness (MINECO) and the Pro CNIC Foundation, and is a Severo Ochoa Center of Excellence (MINECO award SEV-2015-0505).

T-2003

CHARACTERIZATION OF HUMAN OVARIAN CELLS ISOLATED WITH FACS TARGETING DDX4

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The presence of oogonial stem cells (OSC) capable of forming functional follicles with oocytes in the adult human ovary has been reported. These putative stem cells are purified from human ovaries by FACS targeting DDX4, a germ line marker usually expressed in the cell cytoplasm. Following the published protocol, we have isolated a "DDX4 positive" and negative cell populations from ovarian tissue donated by Caesarean section patients. The cells in our hands do not express DDX4 at an RNA level, neither do they form follicles when xenografted into immunodeficient mice within human ovarian cortical pieces. In spite of these negative results, our FACS titration experiments show that the antibody recognizes a specific cell population, and our current experiments are aiming at characterizing these cells. We compared isolated "DDX4 positive" cells to the negative population using single cell RNA sequencing. The expression of 616 genes differed significantly between the two cell populations. Gene ontology analyses suggest that "DDX4 positive" cells express genes related to angiogenesis and cell motility while genes relevant for cell division and proliferation are significantly downregulated when compared to DDX4 negative cells. We are currently carrying out functional experiments for testing the differentiation potential of these cells. In addition, we are evaluating whether the source of human ovarian tissue (transgender versus Caesarean section patients) might affect the results. Altogether, we are working on a thorough characterization of the "DDX4 positive" cells that are already used in experimental fertility treatments at certain clinics.

PLURIPOTENCY

T-2005

PREPARATION OF EUTCD-GRADE HUMAN EMBRYONIC CELL LINES FOR USE AS STARTING MATERIALS FOR THE DEVELOPMENT OF CLINICAL THERAPIES

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The UK Stem Cell Bank (UKSCB) is a key component of the UK regenerative medicine infrastructure charged with procuring, processing (banking and testing) and distributing seed stocks of human embryonic stem cell (hESC) lines for research and human application. One of the key objectives of the UKSCB is to bank and release a panel of carefully selected stem cell lines that meet the EU Tissue and Cell Directives (EUTCD) as set out in Human Tissue Authority (HTA) regulations. These directives set a benchmark for the standards that must be met when carrying out any activity involving tissues and cells for human application. All cells are subject to ethical scrutiny by a national Steering Committee, following which the UKSCB performs a due diligence protocol which establishes for each individual cell line, whether it could meet the requirements of the EUTCD and is thereby provide a starting material for clinical trials. Currently, 39 hESC lines have been approved for deposit as EUTCD-Grade by the UK Steering Committee and at present, 3 lines have been banked and are currently undergoing rigorous testing to ensure future release of seed stocks that are well-characterised and of high quality. Here we describe the key elements of the UKSCB process for these lines including review, processing, characterisation, storage and distribution; all of which are designed to meet the requirements of the UKSCB's regulatory licence which has been maintained successfully for more than 10 years. This current programme of work represents the start of a pipeline of EUTCD-grade hESC lines to support the regenerative medicine community in the development of quality and safety-assured cell therapies. These cell lines are now available for research or commercial development internationally, under a non-exclusive license.

T-2007

POST-TRANSCRIPTIONAL CHARACTERIZATION OF GENETICALLY REGULATED HUMAN NODAL SPLICE VARIANTS

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Nodal and related ligands are highly conserved members of the TGF-beta superfamily with well-established and essential roles in the early embryonic development of vertebrates, and in cell fate decisions in human embryonic stem (hES) cells. Our discovery of a novel human NODAL splice variant suggests that NODAL biology is more complex than is currently appreciated. This splicing event was found to be genetically regulated by a single nucleotide polymorphism, highlighting divergent NODAL biology between individuals as well as between pluripotent stem cell lines used to model early development. The novel transcript contains an alternatively spliced cassette exon that disrupts the constitutive NODAL reading frame. At the protein level, this alternatively spliced NODAL differs from constitutively spliced NODAL in the C-terminal half of the mature peptide. We therefore investigated how the C-terminal region impacts the regulation and function of the NODAL proteoforms. The NODAL variant protein was preferentially secreted relative to constitutively spliced NODAL, but displayed similar extracellular stability and processing dynamics. Differential post-translational modification was partially responsible for this increased secretion, and for NODAL secretion in general. Unlike the constitutively spliced NODAL, the NODAL variant protein is unlikely to adopt a TGF-beta/NODAL-like structure, did not induce expression of targets of canonical NODAL signaling in the zebrafish embryo, and interfering with its splicing in hES cells did not result in decreased expression of markers of pluripotency. In summary, this work demonstrates previously unknown complexity governing human NODAL gene expression and function. These molecular details will help enrich our understanding of NODAL in models of early embryonic development.

T-2009

TFAP2C IS A KEY TRANSCRIPTIONAL REGULATOR OF THE HUMAN NAIVE STATE

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Naïve human embryonic stem cells (hESCs) are specially cultured hESCs that largely recapitulate the transcriptional state of the epiblast of the pre-implantation blastocyst, in contrast to conventionally cultured "primed" hESCs that more closely resemble post-implantation epiblast. Naïve hESCs thus reflect the earliest human developmental state that can be perpetuated in tissue culture. We mapped open chromatin in naïve and primed hESCs and found enrichment of the AP2 transcription factor binding motif at naïve-specific open chromatin. We determined that the AP2 family member TFAP2C is highly upregulated in naïve hESCs and is widespread at naïve-specific enhancers and promoters. Upon culture in naïve conditions, TFAP2C-deficient hESCs rapidly differentiate, indicating that TFAP2C is essential for the human naïve pluripotent state, in contrast to naïve murine ESCs where TFAP2C is non-essential. Human TFAP2C promotes pluripotency and epithelial gene expression, suppresses neural differentiation, and may directly regulate the critical pluripotency factor POU5F1 (OCT4) via a human naive-specific enhancer. Together these results indicate important distinctions between human and murine naïve pluripotency and development.

Funding Source: Broad Stem Cell Research Center; National Institutes of Health; Howard Hughes Medical Institute

T-2011

STRUCTURE-BASED DISCOVERY OF NANOG VARIANT WITH ENHANCED PROPERTIES TO PROMOTE SELF-RENEWAL AND REPROGRAMMING OF PLURIPOTENT STEM CELLS

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NANOG (from Irish mythology Tír na nÓg) transcription factor plays a central role in maintaining pluripotency, cooperating with OCT4 (also known as POU5F1 or OCT3/4), SOX2, and other pluripotency factors. Although the physiological roles of the NANOG protein have been extensively explored, biochemical and biophysical properties in relation to its structural analysis are poorly understood. Here we determined the crystal structure of the human NANOG homeodomain (hNANOG HD) bound to an OCT4 promoter DNA, which revealed amino acid residues involved in DNA recognition that are likely to be functionally important. We generated a series of hNANOG HD alanine substitution mutants based on the protein–DNA interaction and evolutionary conservation and determined their biological activities. Some mutant proteins were less stable, resulting in loss or decreased affinity for DNA binding. Overexpression of the orthologous mouse NANOG (mNANOG) mutants failed to maintain self-renewal of mouse embryonic stem cells without leukemia inhibitory factor. These results suggest that these residues are critical for NANOG transcriptional activity. Interestingly, one mutant, hNANOG L122A, conversely enhanced protein stability and DNA-binding affinity. The mNANOG L122A, when overexpressed in mouse embryonic stem cells, maintained their expression of self-renewal markers even when retinoic acid was added to forcibly drive differentiation. When overexpressed in epiblast stem cells or human induced pluripotent stem cells, the L122A mutants enhanced reprogramming into ground-state pluripotency. These findings demonstrate that structural and biophysical information on key transcriptional factors provides insights into the manipulation of stem cell behaviors and a framework for rational protein engineering.

T-2013

INCREASED SINGLE CELL CLONING EFFICIENCY OF HUMAN PLURIPOTENT STEM CELLS USING A NOVEL CLONING SUPPLEMENT AND MATRIX

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Recent advances in gene-editing techniques such as CRISPR, have led to more accessible and cost effective methods to generate variant human pluripotent stem cell (hPSC) lines for a wide range of research areas. However a major hurdle for gene-editing in hPSCs is the extremely low cloning efficiency of these cells (< 5%), making the generation of clonal cell lines an inefficient process. To address this hurdle we have developed a novel hPSC Cloning Kit comprising a Cloning Supplement and defined Cloning Matrix. To optimize the formulation of our new hPSC Cloning Kit, cells were seeded at a clonal density (25 cells/cm2) in multi-well plates pre-coated with the Cloning Matrix containing mTeSR™1 or TeSR™-E8™ and Cloning Supplement or 10 µM Y-27632. Individual clones were expanded for 7 days and the

resulting colonies in each well were stained for alkaline phosphatase and counted. Cloning efficiencies of 28.1 ± 6.3% and 24.3 ± 6.7% (mean ± SD, n=12) were achieved in mTeSR™1 and TeSR™-E8™ containing the Cloning Supplement, respectively using four independent hPSC lines. This is a significant increase when compared to mTeSR™1 (4.9 ± 2.3%) and TeSR™-E8™ (1.6 ± 1.1%) supplemented with 10 µM Y-27632. The cloning kit was further validated using the more stringent method of single cell deposition of hPSCs into each well of a 96-well plate. hPSCs were dissociated to single cell suspensions and sorted using a BD FACSAria™ Fusion into individual wells (1 cell/well) pre-coated with Cloning Matrix and containing 100 µL of mTeSR™1 with Cloning Supplement or 10 µM Y-27632. Cloning efficiency was significantly higher across all cell lines tested (H1: 18.9%, H7: 15.8%, WLS-1C: 17.9% and STiPS-M001: 27.4%) compared to control mTeSR™1 containing 10 µM Y-27632 (H1: 2.1%, H7: 3.2%, WLS-1C: 5.3% and STiPS-M001: 3.2%). Five H1 and WLS-1C subclones were manually picked and expanded in standard mTeSR™1 for 5 passages. All clonally established hPSC lines displayed similar morphology, expansion rates and proportions of undifferentiated cells compared to the non-clonal control hPSC lines. In summary, this novel hPSC Cloning Kit improves the single cell cloning efficiency of hPSCs which can advance gene-editing studies by facilitating the rapid and successful generation and establishment of new and clonal hPSC cell lines required for those studies.

T-2015

NAÏVECULT - T2ILGÖ MEDIA SYSTEM FOR THE GENERATION AND MAINTENANCE OF CHEMICALLY-INDUCED RESET NAÏVE HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) are traditionally captured in a primed pluripotent state. Recently, the laboratory of Austin Smith, University of Cambridge, developed a transgene-independent method to chemically-induce primed hPSCs to a reset naïve hPSC state. NaïveCult™-t2iLGö is a defined media system developed to support reproducible chemical-induction of the human naïve reset phenotype and the continous robust expansion of reset naïve hPSCs. Chemical generation of reset naïve hPSCs using NaïveCult™ Induction Kit involves sequential steps in which hPSCs, maintained in mTeSR™1 or TeSR™-E8™, are first exposed to a histone deacetylase inhibitor and then subsequently transitioned and maintained in NaïveCult™ Expansion Medium. This process is carried out on hPSCs seeded on inactivated murine fibroblasts and under 5% oxygen conditions. During the transition to reset naïve hPSCs, early passage colonies undergo robust morphological changes characterized by the acquisition of a domed phase-bright morphology on a background of heterogeneous cellular differentiation. By passage 5, cultures become increasingly homogenous with colonies typically demonstrating uniform domed and phase-bright morphology and low levels of background differentiation. Using our optimized protocol and defined media, we generated multiple reset hPSC lines (n=5) from primed human embryonic stem cell lines Shef6, H1 and H9 and human induced pluripotent stem cells WLC-1C and STiPS-F016. These reset hPSC lines, in addition to displaying a naïve cellular phenotype, also demonstrate the expected signature gene expression profiles associated with naive pluripotency. We also tested the ability of reset naïve hPSCs maintained in NaïveCult™ Expansion Medium to differentiate into endoderm, mesoderm and ectoderm by using the STEMdiff™ Definitive Endoderm Kit, STEMdiff™ Mesodermal Induction Medium and STEMdiff™ Neural Induction Medium kits, respectively. Our results demonstrate that these cells are capable of differentiation to all somatic cell lineages with optimal results obtained following a minimum of 21 days of re-priming in TeSR™-E8™ or mTeSR™1. In summary, we have demonstrated robust establishment and expansion of transgene-independent reset naive hPSCs using NaïveCult™ Induction Kit and Expansion Medium.

Funding Source: This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No [602423].

T-2017

GENERATION AND CHARACTERIZATION OF NAIVE HUMAN PLURIPOTENT STEM CELLS (PSCS)-SPECIFIC MONOCLONAL ANTIBODIES BY DECOY IMMUNIZATION

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Pluripotent stem cells exist as two distinct cell states: ESCs (embryonic stem cells) and EpiSCs (epiblast

stem cells) in mouse. ESCs are derived from inner cell mass (ICM) of pre-implantation blastocyst, referred to as naïve pluripotrency, as they retain several molecular characteristics of the ICM. EpiSCs are derived from post-implantation epiblasts, which are referred to as primed pluripotency, and share molecular and functional properties with human PSCs and iPSCs. Interestingly, the different pluripotent states can be interconvertible by transgene (Nanog and Klf4)-dependent or -independent growth conditions with 2iL (PD0325901, CHIR99021 and LIF) or chemicals for inhibitors of signaling pathways. Recently, several groups have also described culture conditions for maintaining naïve hPSCs that share many properties with mESCs. However, the conversion conditions for naïve hPSCs are still controversial. In order to identify and characterize the cell surface molecules and signaling networks that are critical for the maintenance of naïve pluripotency of hPSCs, we generated a panel of murine monoclonal antibodies (MAbs) specific to the naïve state of hPSCs by decoy immunization. First, we converted primed H9 hESCs into the naïve state by using three conditions (1) 2iLAF (2iL, Ascorbic acid and Forskolin in KO-DMEM medium), (2) 2iLLPAF (2iL, LPA and Forskolin in mTeSR1 medium) or (3) 2iLXAV (2iL and XAV939 in DMEM/F12 medium) and investigated expression levels of naïve genes by qRT-PCR. Naïve H9 cells revealed significant upregulation of naive genes such as KLF2/4/5, STELLA, PRDM14, ESRRB and REX1 and significant downregulation of primed genes such as OTX2, SOX17 and DNMT3B. Ten Balb/c mice were then immunized in the right footpads with the primed H9 cells as immunogenic decoys and in the left footpads with the naïve H9 cells as target antigens. After immunization, the left popliteal lymph node cells were collected and were fused with mouse myeloma cells. This fusion resulted in hybridomas which secret a panel of MAbs. Of the MAbs, some MAbs showed strong binding activities to the naïve H9 cells, but only weakly or not at all to the primed H9 cells, suggesting that the MAbs recognize specific molecules in the naïve hPSCs. The isolation and characterization of each MAb-specific molecule is now under investigation.

Funding Source: National Research Foundation of Korea (NRF 2016918220)

T-2019

FUNCTIONAL IDENTIFICATION OF CHEMICAL REGULATORS OF THE EARLY HUMAN BLASTOCYST STATE

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Conventional culture of human embryonic stem cells (hESCs) represents a 'primed' state, which corresponds to the in vivo post-implantation epiblast. While there are multiple publications hitherto describing methods to derive naïve-like hESCs, none of them truly and stably reflect the properties of the in vivo pre-implantation epiblast. Ground-state pluripotency, as defined by its independence from extrinsic signaling, has also been elusive in human since all current protocols still rely on feeders. To tackle this issue, we undertook a high-throughput chemical screen to search for compounds that can maintain naïve-like hESCs in the absence of feeders, using an early blastocyst-specific retroviral reporter. By using the hits to augment our culture medium, we have created a novel formulation that not only supports naïve-like hESCs without feeders, but also shifts the hESCs' expression profile even closer to the in vivo pre-implantation epiblast compared to our previous protocol. Application of these compounds on other naïve-like culture conditions also enabled long-term maintenance of the state in the absence of feeders. Finally, we found that addition of these compounds improves factor-induced reprogramming, extending its utility to induced pluripotent stem cell derivation.

Funding Source: Agency of Science, Technology and Research, Singapore

T-2021

DOWNREGULATION OF ATF1 TRIGGERS NEUROECTODERM DIFFERENTIATION IN HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESCs) are characterized by their robust expansion ability and pluripotency. hESCs maintain pluripotency primarily based on the regulation of the SOX2, OCT4, NANOG transcriptional circuit. Overexpression of SOX2 leads to neuroectoderm differentiation, while overexpression of OCT4 and NANOG induces mesendoderm commitment. However, the upstream transcriptional factor(s) that block SOX2 from overexpression and neuroectoderm determination remain elusive. By a high-throughput screen, we reveal a novel regulator called activating transcription factor 1 (ATF1), which is critical for maintaining pluripotency. The knockdown of ATF1 expression significantly upregulates the neuroectoderm genes but represses the mesoderm, endoderm, and trophectoderm genes. In undifferentiation medium (conditional medium) that supports ESC renewal in the undifferentiated state, downregulation of ATF1 with shRNA or siRNA is sufficient to upregulate PAX6 and SOX2 expression

levels as early as day 2 (1.5-20-fold). Consistently, in the differentiation medium, downregulation of ATF1 promotes neuroectoderm differentiation. In addition, ATF1 knockdown promotes the neuronal differentiation in 3D organoids, which displayed separation of the rudimentary cerebral organization. On the other hand, overexpression of ATF1 suppresses neuroectoderm differentiation and SOX2/PAX6 expression. Corresponding to the potential ATF1 role as an earlier neuroectoderm regulator, we also detected that ATF1 expression was spontaneously downregulated since 24-36 hours upon the neuroectoderm differentiation. Here, our results suggested ATF1 is an important upstream repressor for the expression of SOX2 and PAX6. Importantly, our findings not only explore a novel function of ATF1 in the stem cell state but also reveals the very early step of neuroectoderm differentiation of hESCs.

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T-2023

COORDINATION OF GENE EXPRESSION AND CELL PROLIFERATION BY ACYLGLYCEROL KINASE (AGK) REGULATES PLURIPOTENCY AND REPROGRAMMING

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Embryonic stem cell (ESC) self-renewal and somatic cell reprogramming require the precise coordination of transcription factors, chromatin regulators, and RNA modifiers to be sustained. Many efforts have been devoted to characterizing the mechanism controlling pluripotency. However, the mechanisms maintain the actions ESC self-renewal and undifferentiation remain poorly understood. Acylglycerol kinase (AGK) acts as a lipid kinase, is reported as an important marker in cancer generation, which is related to cell proliferation and cell apoptosis. In this study, we demonstrated that AGK was significantly enriched in human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) compared with HFFs. We also showed that the expression level of AGK gradually decreased in embryoid bodies (EBs), along the course of differentiation, which correlated with the decrease of NANOG and OCT4.In order to further reveal the function of AGK in stem cells, we conducted loss-of-function assays by using two distinct AGK shRNAs that exhibited at least 75% knockdown of AGK in ESCs, it showed that the ability of cell proliferation was decreased by impaired cell-cycle process. And loss of AGK presented derverse differentiate fate compared to WT ES cells. Meanwhile, we examined AGK's function during iPSC reprogramming. HFFs were transfected with OCT4, SOX2, KLF4, c-MYC (OSKM) with or without AGK by lentiviral system. The results showed that upregulation of AGK during human iPSCs generation enhanced the number of iPSC colonies. Taken together, these results indicate that AGK plays a vital role on maintaining the pluripotency state of ESCs and promoting the efficiency of iPS regrogramming process.

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T-2025

MAINTENANCE OF MAMMALIAN STEM CELL STATES BY HONEY BEE ROYAL JELLY

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Royal jelly is the well known queen-maker for the honey bee Apis mellifera, and has documented cross-species effects on longevity, fertility, and regeneration in mammals. How Royal jelly or its components exert their myriad effects has however remained poorly understood. Here we show, for the first time, novel wound healing and stemness maintenance activities for Royal jelly, in mice and in embryonic and post-natal stem cells, respectively. Royal jelly contains a robust activity that activates a pluripotency gene regulatory network through control of chromatin dynamics to effect a phenotype that mimics ground state pluripotency. Thus, our findings implicate the existence of factor(s) in Royal jelly that can maintain cellular identity through affecting the dynamic state of stem cells in non-insect species, uncovering an important heretofore unappreciated innate program for stem cell self-renewal with broad implications in understanding the molecular regulation of stem cell fate.

Funding Source: Burroughs Wellcome Fund

T-2027

GENERATION OF FULL-TERM DEVELOPMENT OFFSPRING WITH RAT EMBRYONIC STEM CELLS VIA TETRAPLOID COMPLEMENTATION

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Functional evaluation of the developmental potency of embryonic stem cells (ESCs), which varies across species depending on the derivation and culture conditions, is a prerequisite for understanding the pluripotency. Tetraploid (4n) complementation, through which the ESCs produce an entire organism, is considered to be the most stringent test of developmental potency. It remains unclear whether ESCs from species other than mice can pass the test to date. Here we report the establishment of the tetraploid complementation assay in rats. Though the assay we show that the rat ESCs at very early passages are also capable to produce live animals, however, this capacity is severely compromised during cell culture due to the genome-wide demethylation-coupled loss of genomic imprinting. Our findings support the existence of ground state of pluripotency in rats, and points to that the maintenance of ground state pluripotency may vary between species.

T-2029

EPIGENETIC VARIATIONS AT IMPRINTING CONTROL REGIONS ARE DEPENDENT ON CLONE AND GENE LOCI BUT NOT ON PLURIPOTENT STATES

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Induced pluripotent stem cells (iPSCs) can be generated by enforced expression of transcription factors such as Oct3/4, Sox2, Klf4 and c-Myc in somatic cells. Dynamic epigenetic alterations occur during the reprogramming process. In order to achieve medical applications using iPSCs, it is important to understand epigenetic variations (e.g., genomic imprints) of iPSCs, which potentially affect characteristics of iPSCs. Here, we examined the stability of genomic imprints in various pluripotent stem cells by MethylC-seq analysis and bisulfite sequencing. We first established naïve ESCs, iPSCs and primed induced epiblast stem cells (iEpiSCs) derived from F1 mouse embryos between 129/Sv and MSM/Ms genetic background to discriminate parental alleles by SNPs. We found that Igf2 is transcribed from both alleles in some ESC, iPSC and iEpiSC clones, which is accompanied by the altered DNA methylation at H19 DMR (paternal imprint). In contrast to H19 DMR, DNA methylation status at Peg3, Peg10 and Snrpn DMR (maternal imprints) were maintained in most ESCs, iPSCs and iEpiSCs. Comprehensive imprinting analyses revealed that paternal imprints are more unstable rather than maternal imprints during reprogramming process. Furthermore, we found that the altered imprint status of Igf2/H19 is inherited into differentiated cells indicating that that aberrant imprinting persists in somatic cells throughout the lifetime. Collectively, stability of genomic imprints in stem cells is dependent on gene loci rather than pluripotent states (Naïve or Primed). In this presentation, we will discuss the role of altered imprinting regulation on the quality of stem cells based on our recent results in comprehensive imprinting analyses of F1 pluripotent stem cells.

PLURIPOTENT STEM CELL DIFFERENTIATION

T-2031

MODIFIED MRNA-MEDIATED GLYCOENGINEERING AMELIORATES DEFICIENT HOMING PROPERTIES OF HUMAN IPSC-DERIVED HEMATOPOIETIC PROGENITORS

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Pluripotent stem cells (PSCs) have the potential to serve as an inexhaustible cell source to generate hematopoietic stem and progenitor cells (HSPCs) for use in hematopoietic cell production, disease modeling, and eventually transplantation medicine. However, despite much effort in the field, clinically relevant generation of functional HSPCs from human PSCs has not yet been achieved. Bone marrow (BM) homing is an important aspect of HSPC biology that has remained largely unaddressed in efforts to derive functional HSPCs from human PSCs. We therefore examined the BM homing properties of human induced pluripotent stem cell-derived HSPCs (hiPS-HSPCs) and found that they express molecular effectors of BM extravasation

such as CXCR4 and VLA-4, but lack expression of E-selectin ligands which program trafficking to BM. To overcome this deficiency, we ectopically expressed a panel of human fucosyltransferases using modified-mRNA, and found that expression of fucosyltransferase 6 resulted in marked increases in cell surface E-selectin ligands. These "glycoengineered" hiPS-HSPCs exhibited enhanced tethering/rolling interactions on E-selectin-bearing endothelium under flow conditions in vitro and significantly increased BM trafficking and extravasation in the calvarial BM of xeno-transplanted mice in vivo. However, despite marked improvements in homing, glycoengineered cells did not engraft mice beyond 6 weeks post-transplant, indicating that additional functional deficiencies still exist in these cells. Our results suggest that strategies aimed at increasing E-selectin ligand expression could be applicable as part of a multifaceted approach to optimize the production of functional HSPCs from human PSCs.

T-2033

GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED SPINAL PRO-OLIGODENDROGENIC NEURAL PRECURSOR CELLS

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Transplantation of human induced pluripotent stem cell-derived neural precursor cells (hiPSC-NPCs) is an exciting therapeutic strategy for the treatment of CNS diseases including traumatic spinal cord injury (SCI). NPCs are tripotent cells with the ability to differentiate into oligodendrocytes, neurons and astrocytes, however, the proportion of oligodendrocytes generated is low relative to their important role in remyelinating denuded long-tract sensorimotor axons to promote behavioral recovery. We have developed a novel protocol to generate hiPSC-NPCs with a differentiation bias towards an oligodendrocyte lineage by mimicking exogenous morphogenic cues found during embryonic neural tube patterning. The resultant spinal pro-oligodendrogenic cells are tripotent NPCs with a strong predisposition to differentiate to oligodendrocytes. Retinoic acid, a potent caudalizing factor, and sonic hedgehog, a ventralizing morphogen, are used at key stages to drive hiPSC-NPCs to a ventral spinal progenitor fate from days 3-12 in vitro. Subsequent addition of PDGF-AA and Thyroxine directs cells towards a pro-oligodendrogenic phenotype which has been extensively characterized in vitro. When transplanted into a clinically-relevant clip-contusion model of cervical spinal cord injury, both conventionally-derived hiPSC-NPCs and pro-oligodendrogenic NPCs had the ability to differentiate into the three major neuroglial lineages, however, hiPSC-NPCs predominantly generated neurons and astrocytes while hiPSC-pro-oligodendrogenic-NPCs largely differentiated to mature remyelinating oligodendrocytes. This represents key proof-of-concept data that our protocol can generate pro-oligodendrogenic-NPCs which retain their unique phenotype in vitro and in vivo. This has important implications for several CNS disorders where demyelination is a substantial cause of neurological deficit.

Funding Source: Ontario Institute for Regenerative Medicine, Krembil Research Fundation, Wings for Life Fundation

T-2035

THE THERAPEUTIC POTENTIAL OF THE MELANOCYTES DERIVED FROM PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS IN AUTOLOGOUS TRANSPLANTATION

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Induced pluripotent stem cells (iPSCs) have the potential to differentiate into melanocytes which are functional in vitro. Compared to other potential sources, iPSCs can be established from patients and they can also propagate indefinitely. Therefore, iPSCs could provide an unlimited source of autologous melanocytes for patients with depigmentation. However, the in vivo function of these melanocytes remains unknown and whether they could be applied in the cellular transplantation and therapeutics need to be clarified. Here, we established vitiligo patient-specific iPSCs and generated melanocytes using a novel 3D differentiation system. To investigate the vivo function, we modified a hair follicle reconstitution assay and transplanted these melanocytes into immunodeficiency mice intracutaneously. The results showed that compared with the conventional flat culture, the newly established 3D cultural system improved the differentiation efficiency and helped to generate large quantities of melanocytes using patient-specific iPSCs. These induced melanocytes were equivalent to normal human epidermal melanocytes (HEM) in vitro, including melanocytic markers expression and melanosome generation. RNA-sequencing analysis also suggested the significant upregulation of representative genes involving in melanogenesis. More importantly, we found iPSCs-derived melanocytes contributed to generate pigmented hair follicles and the integration efficiency was higher than HEM. In the reconstituted

hair follicles, both Fontana–Masson staining and DOPA staining showed clearly that pigmentation was mainly localized in the hair bulb and hair shafts. Furthermore, the immunofluorescent co-expression for both human nuclei specific recognizing antibody and melanocytic marker TYRP1 suggested the melanin was produced by the human induced melanocytes in the hair bulb where was the normal location of mature melanocytes in mice and human. Together, our findings demonstrate that induced melanocytes from vitiligo patient-specific iPSCs are functional both in vitro and in vivo and it is the first time in this study to identify their in vivo function. It should provide a potential source for personalized cellular therapy for patients with depigmentation and also it could help to explore the detailed pathogenesis.

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T-2037

PROCESS DEVELOPMENT FOR A SCALABLE PRODUCTION OF HUMAN PLURIPOTENT STEM CELL-DERVIED CARDIOMYOCYTES WITH THE BAG-BASED BIOREACTOR

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We previously have successfully established a scalable and cGMP-compliant process for large-scale production of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) in suspension culture using spinner flasks at a scale up to 3 liters. While this stirred-base suspension platform was able to produce hPSC-CMs with purity >90% for several cell lines, we noticed that shear or mechanical stress generated by stirring may affect production performance for some sensitive cell lines. Alternatively, a rocking suspension platform, such as bag-based bioreactor, provides advantage of homogeneous and mild shear force at agitation that may minimize detrimental effects on those sensitive cell lines. To test if the advantage of the rocking platform would benefit cardiac differentiation in suspension, the same differentiation procedure was performed on a shear-sensitive cell line with a spinner flask and a T flask on a rocking platform. The result showed that cardiac differentiation conducted in the rocking suspension culture exhibited better cell yield and purity. The scalability of the rocking suspension culture was then further tested in the bag-based suspension system. By optimizing the critical parameters, including rocking conditions, cell aggregate size, concentration and induction timing of cardiac inducers, for the cardiac differentiation in suspension culture, we were able to generate hPSC-CMs with an average yield of 1.5 to 2.1 × 106 CM/mL and >90% purity using the bag-based bioreactor at a scale up to 3 liters. With the optimized differentiation condition, control of gases, pH, and temperature, and monitoring of glucose and lactate levels, we demonstrated scalability and consistency of the bag-based bioreactor in production of hPSC-CMs. In summary, we have developed a robust and scalable hPSC-CM manufacturing process with the bag-based bioreactor. The rocking suspension culture system provides an alternative platform for large-scale production of hPSC-CMs for those cell lines sensitive to shear or mechanical stress. The rocking-based suspension culture system we shown in this study, together with the stirred-based suspension system we developed previously, will accelerate the application of various hPSC lines for pre-clinical and clinical studies.

Funding Source: CIRM (California Institute for Regenerative Medicine)

T-2039

PSC-DERIVED HUMAN TELENCEPHALIC NEURONS REACH SYNAPTIC MATURITY AND ELICIT SPONTANEOUS ACTIVITY

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Human pluripotent stem cells (hPSCs) are a powerful tool for studying human neurodevelopment in health

and disease, drug screening and cell therapy. Here we present a detailed description of a feeder-free telencephalic differentiation protocol from hPSCs. An induced hPSC line (33Q#1) and an embryonic hPSC line (Genea 19) were used in the present study. Gene expression was analysed at day in vitro (DIV) 0, 8, 12, 16, 23 and 37 of differentiation by OpenArray. We assessed differentiation progression by immunostaining for pluripotent, neuroectodermal, neural progenitors and neuronal markers. We also analysed neuronal activity by single-cell calcium imaging at DIV 37 and spikes' properties were inferred by a customized-MATLAB software. We found that hPSCs exit from pluripotency and commit to a neuroectodermal fate by DIV 4 and 8 respectively. By DIV 16, hPSC-derived neuroepithelial cells mainly express subpallial markers (DLX+ and EBF1+), although some progenitors expressing pallial markers (PAX6+) were also detected. By DIV 37, most cells mature into postmitotic MAP2B+ (94.5%) neurons that form neuronal networks consisting of glutamatergic (Tbr1+), dopaminergic (TH+) and GABAergic (GABA+) neurons, including a sub-population of striatal projection neurons (DARPP-32+/CTIP2+). Moreover, the defined neuronal maturation medium induced the expression of synaptic genes, including Glutamate-, GABA-, SP-, Glycine-, Serotonin- and ATP-ion-gated receptors and a battery of K+, Na+, Ca2+ and Cl- ion/voltage-gated channels. Furthermore, calcium imaging revealed that 84% of neurons elicited spontaneous events with heterogeneous firing patterns which were segregated in eight differential k-means groups based on nine defined neuronal features. Neonatal mouse striatal transplantation of hPSC-derived neuronal progenitors demonstrates that striatal environmental cues promote neuronal progenitor survival, differentiation and integration into the endogenous circuitry with hPSC-derived neurons selectively projecting to the Globus Pallidus. In conclusion, we present a novel and fast feeder-free protocol for the differentiation of hPSCs to mature and physiologically active telencephalic neurons in 37 days which is useful for human disease modelling, drug screening and cell therapy applications.

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T-2041

GENERATION OF LUNG ORGAN FROM EMBRYONIC STEM CELLS VIA BLASTOCYST COMPLEMENTATION IN MICE

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Generation of the organ has been extensively researched using embryonic stem cells (ES cells) and induced pluripotent stem cells (iPSCs). It has been reported that generation of pancreas and kidney from those cells via blastocyst complementation. But to our knowledge, there has been no report about lung generation. The fibroblast growth factor 10 (Fgf10) is a gene involved in development of limb and lung in mice. The Fgf10-/- mice presents a phenotype of limb and lung deficiency. Therefore, we tried to generate the lung organ from embryonic stem cells via blastocyst complementation. Previously, we have developed Fgf10-/- mice by CRISPR/Cas system (Scientific Reports 4:5705). Since the Fgf10-/- die immediately after birth, the Fgf10+/- genotype mice are maintained. These Fgf10+/- mice were mated, and Fgf10-/- embryos were obtained. When Fgf10-/- embryo developed into blastocysts, enhanced green fluorescent protein (EGFP) positive murine ES cells were injected in it. Theses blastocysts were implanted into pseudopregnant mice. We observed presence of the lung of the mice by fluorescent stereomicroscope, H-E staining and immunofluorescence.We implanted 40 control embryos that was not injected ES cells into pseudopregnant mice and we obtained 13 littermates (32.5%) from the mice. 4 littermates (30.7%) showed limb defect (Fgf10-/-) and they were confirmed histologically that they had hypoplastic or aplastic lung. 268 embryos injected ES cells were made and 253 of

them were transferred into pseudopregnant mice. 45 littermates (17.8%) were obtained from those mice. 38 littermates were EGFP positive at time of birth and 20 littermates (44.4%) showed ventral hernia and died. All mice died at birth (20 mice) showed the lung organ histologically. We sacrificed and analyzed histology in EGFP positive mice over one month. EGFP was positive in their lungs by fluorescent stereomicroscope. The tissue, cells, and structure of their lung were well developed and not different from normal mouse lung by H-E staining. Frozen section of the lung showed EGFP positive in alveolar epithelium, endothelium, tracheal cartilage. ES cells can be able to generate lung by blastocyst complementation in lung aplasia mice model.

T-2043

GENETIC AND FUNCTIONAL INVESTIGATION OF EPISODIC ATAXIA 1

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Bioelectrical signals generated by ion channels play crucial roles in excitation genesis and impulse conduction in excitable cells as well as in cell growth, migration and apoptosis in proliferative cells. Episodic Ataxia 1 (EA1) is a rare autosomal dominant neurological disorder and was the first ionic channel disease to be associated with defects in a potassium channel, Kv1.1. The disease affects both central and peripheral nerve functions, giving symptomatic attacks of imbalance and uncontrolled movements. Directed differentiation of human induced pluripotent stem cells into functional, region-specific neural cells is a key step to realizing their therapeutic promise to treat various neural disorders including EA1. To determine the neuronal consequences of Kv1.1 mutations, we used somatic cell reprogramming to generate iPSCs from the skin fibroblasts of individuals with EA1. We next differentiated the iPSC lines into neuronal precursor cells (NPCs) and subsequently neurons using conditions that favor the generation of cortical neurons. A total of 12 stable clones for the corresponding 6 cell lines were produced. The colonies were initially selected based on having a similar morphology to ESCs. Next the standard assays were performed, namely detection of pluripotency markers (through RT-qPCR and/or immunostaining), confirmation of absence of episomal plasmid and cytogenetic analysis. IPSCs were then differentiated into cortical precursor neurons and the presence of markers specific for neuronal cortical identity were assessed by qPCR and imunoflorescence, which confirmed the successful conversion. These were differentiated to neurons using forced expression of Ngn2 and we have preliminary data to suggest maturation of the cells. Future applications for iPSC-derived neurons of EA1 beyond modelling include drug testing and the potential to investigate the mutagenic diversity of encountered in EA1 patients.

T-2045

GENERATION OF HIGHLY PURE SPINAL MOTOR NEURONS FROM HUMAN PLURIPOTENT STEM CELLS FOR AMYOTROPHIC LATERAL SCLEROSIS AND SPINAL MUSCULAR ATROPHY MODELING

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Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are progressive neurodegenerative disorders characterized by the gradual degeneration and elimination of motor neurons (MNs) and skeletal muscles leading to paralysis, respiratory insufficiency, and death. Human pluripotent stem cells (hPSCs) are a promising source of disease-affected differentiated cells and have opened new prospects for understanding human development, disease modeling and mechanisms, and therapeutic development. We hypothesize that directed differentiation of hPSCs into MNs in vitro using development related small molecules is a useful way to study cellular and molecular mechanisms of these fatal neurodegenerative diseases and develop novel strategies for curing ALS and SMA. However, this approach is hindered by the low yield of MNs and heterogeneity of the cell populations generated and suboptimal characterization. In this study, by using a combination of small molecules, we instruct hPSCs and generate high-yield of spinal motor neuron progenitors (MNPs) and mature and functional MNs. Efficient derivation of spinal MNs from hPSCs will provide invaluable tools to facilitate in-depth study of these diseases and identification of human cell-relavant therapeutic targets.

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T-2047

EPIGENOMIC CONTROL OF PLURIPOTENT STEM CELL SELF-RENEWAL AND DIFFERENTIATION: INSIGHTS FROM REGULATION OF THE ATP-BINDING CASSETTE TRANSPORTER SUPERFAMILY

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There are about 48 ATP-binding cassette (ABC) transporter genes in mammalian genomes, which constitute a superfamily with diverse functions on cell membrane and play critical roles in both physiological and pathological sittings. However, the roles of ABC transporters in pluripotent stem cells have not been well elucidated. In this study, we used an integrated approach to investigate ABC transporter expression and function in different cellular states of pluripotent stem cells. We found that human pluripotent stem cells expressed at least 45% of ABC transporters such as ABCA1, ABCB6, ABCC1, ABCD3, ABCF1, and ABCG2. Moreover, ABC expression patterns were associated with their DNA methylation status, particularly in ABCA, ABCD, ABCG subfamilies (Pearson coefficient $r > 0.60$). Currently, we are employing various DNA methyltransferase (DNMT or Dnmt) deletion mutants derived from both human and mouse embryonic stem cells (hESCs and mESCs respectively) to determine the role of DNA methyltransferases in the regulation ABC transporter expression in these cells with pluripotent and differentiation states. In an initial study of mESCs, we found mouse Dnmt-mediated methylations in mESCs decreased expression of a cluster of abc genes, particularly for Abcb1b, Abcb6, Abcc1, and Abcg2. Concomitantly, Dnmt-mediated methylations also enhanced expression of a group of microRNAs. Collectively, our data indicate that Dnmt-mediated methylations might inhibit Abc expression patterns via a boosted microRNA pool that involves miR-326, miR-328, miR-345, and miR-1291. Now, we are confirming these results in hESCs with Dnmt deletions. Furthermore, global methylation-associated mRNA transcriptomes are linked to a whole-genome response to small chemical drugs as predicated by the Connectivity Map (cMap) that aims to bridge the functional connections among drugs, genes and diseases. We are also validating the predicated functions in 20 projected compounds with the highest cMap scores by cheminformatics analysis and pharmacological experimentation. Thus, the stage-specific surface and membrane expression of pan ABC transporters might imply their miscellaneous functions (such as lipid transporter, detoxification, and homoeostasis) in cells or tissues at different mammalian developmental stages.

T-2049

ANALYSIS OF EXPRESSION PROFILE OF HAND1 AND HAND2 IN EARLY CARDIOGENESIS USING HIPSCS

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There are a lot of representative transcription factors of human cardiac development and the network of cardiac transcription factors is quiet complicated. It is known that cardiac differentiation from iPSCs recapitulates cardiac development using iPSCs. Here we established HAND1 and HAND2 double reporter iPSC to get clear insights of HAND1 and HAND2 during cardiac differentiation. The absent of HAND1 or HAND2 in mice causes left and right ventricular hypoplasia respectively, which indicates HAND1 and HAND2 are essential for heart development. In addition, heterozygous deletion of HAND1 or HAND2 in human iPSCs shows less efficiency of cardiomyocyte differentiation. We observed that HAND1 is expressed all cardiovascular progenitor cells after mesodermal stage and some of HAND1 positive cells became negative. HAND2 is expressed from some of HAND1 positive cells after cardiovascular stage. For long time culture, there are HAND1 or HAND2 single positive cardiomyocytes and HAND1 and HAND2 double positive cardiomyocytes. Now we investigating when the lineage commitment is determined and which factors contribute to cardiac lineage with HAND1 and HAND2 using gene expression analysis and lineage tracing system.

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T-2051

INVESTIGATING THE ROLE OF SFTA3 IN THE SPECIFICATION OF INHIBITORY INTERNEURON CELL FATE

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During embryonic development of the mammalian telencephalon, the transcription factor NKX2.1 is highly expressed in the medial ganglionic eminence (MGE), a transient structure of the ventral forebrain that is a major source of cortical GABAergic inhibitory interneuron progenitors. Utilizing transcriptome analysis of human embryonic stem cell (hESC)-derived MGE-like interneuron progenitors to identify additional gene candidates with expression profiles similar to NKX2.1, we identified SFTA3, which encodes a surfactant protein SP-H, as the strongest candidate. Quantitative real-time PCR analysis of hESC-derived NKX2.1+ progenitors revealed a similar increase in expression of NKX2.1 and SFTA3 at various time points during interneuron differentiation. Examination of transcriptome data available from the Allen Institute for Brain Science also illustrates high SFTA3 gene expression in the human MGE. Although SFTA3 has been well studied in the lung, the possible role of this surfactant protein in the MGE during early embryonic development remains unexamined. To determine if SFTA3 plays a role in MGE specification, SFTA3-/- and NKX2.1 -/- hESC lines were generated using custom designed CRISPRs. Previous studies have demonstrated NKX2.1 deficiency results in diminished or complete absence of MGE derivatives. Therefore, we expect NKX2.1 knockout and perhaps SFTA3 knockout ESC lines will demonstrate a diminished capacity to differentiate into MGE-derived interneuron subtypes. Long term in vitro maturation of neural progenitors derived from SFTA3 and NKX2.1 knockout and control cell lines are currently ongoing to examine the role of SFTA3 in specifying GABAergic interneuron subtypes. Recent studies, including immunohistochemistry and protein structure analysis, suggest that SP-H is present at the plasma membrane. This suggests that hESC-derived NKX2.1+ interneuron progenitors can be enriched using a fluorophore conjugated SFTA3 antibody via fluorescence activated cell sorting. This would facilitate isolation of MGE-like progenitors from non-genetically modified cells, making them suitable for clinical application.

Funding Source: Connecticut Regenerative Medicine Research Fund

T-2053

ENRICHMENT OF PLURIPOTENT STEM CELL-DERIVED HEPATOCYTE-LIKE CELLS BY LANFORD MEDIUM AND LOW-MOLECULAR COMPOUND

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Human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have the potential to become an effective resource for the regeneration of defective organs, including the liver. However, undifferentiated cells that remain following differentiation have teratoma-forming potential. In addition, practical applications require a large quantity of differentiated cells, so the differentiation process must be economical. In this study, we analyzed global changes of gene expression during the differentiation by DNA microarray using six human pluripotent stem cell lines, human ES cell: khES1 (Kyoto), khES2 (Kyoto), khES3 (Kyoto), H1 (Wisconsin) and human iPS cell: 253G1 (Kyoto), 201B7 (Kyoto) and two somatic cells: human adipose-derived stem cells (Lonza), human fibroblasts. To induce differentiation, we produced spherical multicellular aggregates known as embryoid bodies (EBs) and identified differences and commonalities among six human pluripotent stem cell lines. EBs formed without requiring supplementation with inducing factors. These EBs expressed some liver-specific metabolic genes including the ammonia-metabolizing enzymes glutamine synthetase and carbamoyl-phosphate synthase 1. Real-time PCR analysis revealed increased expression of hepatocyte markers in EBs treated with ammonia in Lanford medium. Analysis of DNA microarray data showed that hepatocyte-like cells were the most abundant population in ammonia-treated cells. Furthermore, expression levels of pluripotent stem cell markers were drastically reduced, suggesting a reduced teratoma-forming capacity. These results indicate that treatment of EBs with ammonia in Lanford medium may be an effective inducer of hepatic differentiation without expensive inducing factors.

T-2055

NANOGROOVED SUBSTRATED PROMOTES DIRECT LINEAGE REPROGRAMMING OF FIBROBLASTS TO FUNCTIONAL DOPAMINERGIC NEURONS

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The generation of dopaminergic (DA) neurons via direct lineage reprogramming can potentially provide a novel therapeutic platform for the study and treatment of Parkinson's disease. Here, we showed that nanoscale biophysical stimulation can promote the direct lineage reprogramming of somatic fibroblasts to induced DA (iDA) neurons. Fibroblasts that were cultured on flat, microgrooved, and nanogrooved substrates responded differently to the patterned substrates in terms of cell alignment. Subsequently, the DA marker expressions, acquisition of mature DA neuronal phenotypes, and the conversion efficiency were enhanced mostly on the nanogrooved substrate. These results may be attributed to specific histone modifications and transcriptional changes associated with mesenchymal to epithelial transition. Taken together, these results suggest that the nanopatterned substrate can serve as an efficient stimulant for direct lineage reprogramming to iDA neurons, and its effectiveness confirms that substrate nanotopography plays a critical role in the cell fate changes during direct lineage reprogramming.

Funding Source: This work was supported by the, Korea Health Technology R&D Project, Ministry of Health & Welfare (HI16C1176).

T-2057

PREDICTING CELL LINE VARIABILITY IN CARDIOMYOCYTE DIFFERENTIATION EFFICIENCY USING NON-INVASIVE MULTI-ANALYTE LUMINEX® ASSAYS

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The ability of pluripotent stem cells to differentiate into any tissue of the body has the potential to revolutionize medicine. One technical challenge that must be overcome to fully realize this potential is generating patient-derived pluripotent stem cells that can efficiently and robustly differentiate into specific tissues. Even using standardized differentiation protocols, differentiation efficiency is highly variable across cell lines from both human embryonic stem (hES) and human induced pluripotent stem (hiPS) cells. In this study, we demonstrate the power of Luminex® Assays as an early detection method to assess pluripotent cell line variability and to determine cell line differentiation efficiency. Various hES and hiPS cell lines were differentiated into cardiomyocytes using the standardized protocol and reagents in the StemXVivo® Cardiomyocyte Differentiation Kit. Combining our standardized differentiation protocol with multi-analyte Luminex® assays enabled us to profile the changes in cytokine and growth factor levels in cell culture media at key stages during the differentiation. We found that cytokine and growth factor expression profiles varied across hiPS cell lines with known differences in cardiomyocyte differentiation efficiency. Because analytic samples are obtained from culture media, the cells are able to continue through the differentiation process and be analyzed for efficiency by assessing beating and cardiac-specific marker expression via immunocytochemistry. We demonstrate that the multi-analyte profile of cell lines with robust differentiation differs from cell lines with lower efficiencies. Using Luminex® multi-analyte technology we identified particular analytes that are predictive of differentiation success. Additionally, this information can be used for identification of important pathways involved in stem cell differentiation and/or maturation into cardiomyocytes.

T-2059

EARLY CELL FATE DECISION MAKING IN ESC DIFFERENTIATION USING SINGLE CELL RNA-SEQ AND LINEAGE TRACING

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Chromatin states guide developmental decisions and contribute to the maintenance of cellular state. We use mouse embryoid body (EB) differentiation as a model to understand how chromatin regulatory processes guide early cell fate decisions. We utilized single cell RNA-seq to profile 1,101 single cells from 8 time points of EB differentiation. We identified all 3 germ layers (Mesoderm, Endoderm, and Ectoderm), and could further annotate several sub-populations representing primitive endoderm (PrE), intermediate stage, primordial germ cells (PGC), EpiSC-like cells, primitive streak (PS), and hemangioblast-like cells. To model differentiation trajectories, we applied an unsupervised algorithm (monocle2), which increases the temporal resolution of transcriptome dynamics. We found that pseudo-time of branching, or the appearance of each sub-population, recapitulates the time line of early development. In addition, we show that the first branching event begins

at the intermediate stages, and trajectories are separated by the differential expression of Dnmt3b and Tet1/2. This suggests that uneven DNA methylation may drive this lineage decision. The first of these early branches (Dnmt3blow/ Tet1/2hi) expressed known marker genes of PGC, recapitulating the DNA hypo-methylation in early embryonic germ cell development. The second branch (Dnmt3bhi/ Tet1/2low) differentiated through EpiSC-like/PS into 3 germ layers. We are applying genetic (CRISPR) perturbations of several target genes differentially expressed at branch points to define their importance for lineage specification. In addition, We are following up the potential importance of specific regulatory elements and chromatin states by epigenomic profiling. Together this work provides an initial snap shot of very early cell fate decisions and begins to hint at the underlying mechanisms.

Funding Source: Centers of Excellence in Genomic Science (CEGS), NHGRI,NIH

T-2061

A STEAMLINED CHEMICAL RECIPE FOR GENERATION OF AUTHENTIC STRIATAL PROGENITORS FROM HUMAN EMBRYONIC STEM CELLS

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Differentiation of human pluripotent stem cells (hPSCs) into striatal medium spiny neurons (MSNs) promises a cure for neural degenerative disorders such as Huntington's disease, premised on the success in robust generation of such population of neurons. Current available procedures of MSN differentiation incline toward suboptimal either in quality, purity, efficiency, or in easiness. Here, we develop a streamlined protocol of MSN production through sequential combination of dual-SMAD inhibition and lateral ganglionic eminence (LGE) patterning with a set of small molecules. Using this protocol, we could efficiently induce human ESCs into MEIS2 expressing LGE-like neural progenitors and DARPP32 expressing striatal MSNs within 14 days and 18 days, respectively. These MSNs can express canonical GABAergic MSN markers authentically such as DARPP32, MEIS2, CTIP2 and FOXP2, and could fire trains of action potentials. Upon transplantation into mouse model of Huntington's disease, they can alleviate the locomotive disorders of the diseased mice. Compared with previously published methods of neural differentiation, we thus invent a rapid and efficient protocol to generate large population of authentic striatal LGE-like neural progenitors from hPSCs with efficiency up to 85% within 14 days, which is valuable for dissecting the fate determination of striatal neurons and cell therapy of MSN degenerative diseases.

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T-2063

DEVELOPING STRATEGIES TO PRODUCE NOTOCHORD LIKE CELLS FROM HUMAN ESC/ IPSC

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Intervertebral disc disease (IDD) leads to low back pain and disability globally. Progressive loss of notochord cells (NCCs) and replaced with hypertrophic chondrocyte-like nucleus pulposus cells (NPCs) are associated with the onset of IDD. Cell-based therapy has been shown the promising for many diseases, including the IDD in preclinical studies. However, the limited availability of human NCC has hurdled such application for disc repair. This study aimed to define strategies to derive human NCCs and NPCs from human ESC/iPSC. hESC3, 9 and imR-90-iPSC were used for a two-step protocol to differentiate into NCCs. Activin A was used in step 1 to prime pluripotency to mesodermal potency. Activin A and Wnt inhibitor were used in step 2 for further notochordal differentiation. Our results showed that NCCs were successfully derived from the above protocol confirmed by immunofluorescence staining of Noto, Brachyury (T) and Foxa2. RT-PCR results showed that the expression of Noto, T and Foxa2 was the highest at day 4 after differentiation. NCCs can be isolated using Noto-smartflare probe and the efficiency was around 20%. Furthermore, these NCCs can be differentiated into NPCs. These NPCs expressed Tie2 (Tie2), disialoganglioside 2 (GD2), collagen II and aggrecan. Co-culturing NPCs with light2 cell line, a luciferase-based reporter responsive to sonic hedgehog (Shh) secreted protein, showed NPCs synthesizes Shh. Moreover, no teratoma was formed at 8 weeks of iPSC/ ESC- NPCs transplantation in the immunodeficient SCID mice, indicating these NPCs is safe for transplantation in vivo. Our study demonstrates that NCCs could be induced from human pluripotent stem cells through regulation of retinoic acid, BMP, FGF and Wnt signaling.

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T-2065

ROBUST DIFFERENTIATION OF HIGHLY PURE CARDIOMYOCYTES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Robust generation of human induced pluripotent stem cells(iPSCs)-derived cardiomyocytes(CMs) is important for drug screening, disease modeling, cell-based therapeutic applications, and investigating developmental mechanisms during cardiac specification. Although numerous protocols have been reported for the derivation of cardiomyocyte-like cells from human ES and iPS cells, the efficiency and reproducibility of these methodologies still remain to be improved. Integration-free iPS cells were generated from human peripheral blood mononuclear cells (PBMC) using the CytoTune®-iPS Sendai Reprogramming Kit following the manufacture's protocol. The properties of the iPSC were confirmed by standard procedure. Then, cardiac differentiation from iPS cells were carried out using a new protocol developed by our company. The iPS cells reprogrammed from PBMCs were positive for pluripotency markers NANOG, OCT4, SOX2 and SSEA4 and exhibited the ability of differentiation to three germ layers. Using these iPS cells, we established an optimal protocol for cardiomyocyte differentiation. More than 90% of the cells were positive for cTNT staining by flowcytometry analysis after 12 days of differentiation. The yield is high as one seeded iPS cell yielded 30 viable cardiomyocytes. Furthermore, the differentiated cells showed high rate of re-attachment after cell dissociation. More than 80% of beating cardiomyocytes re-attached to the Matrigel coated plates, which is critical for downstream experiments. In conclusion, we developed a robust platform for scale-up production of functional iPS cell-derived cardiomyocytes with high purity. These cardiomyocytes are very useful for drug screening and disease modeling.

T-2067

EFFECTIVE GENERATION OF HEPATOCYTE-LIKE CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Prediction of drug-induced liver injury is a critical step for drug screening. Primary human hepatocytes are predominantly used for the prediction of drug-induced liver injury currently. However, the limitation of the availability of the primary human hepatocytes is one of the major obstacles for toxicity screening. Therefore, there is an urgent need for developing novel and efficient approaches to produce hepatocytes. Here we reported an effective protocol for generating human hepatocyte-like cells from induced pluripotent stem (iPS) cells for drug metabolism study. Integration-free iPS cells were generated from human peripheral blood mononuclear cells(PBMCs). The properties of the iPSC were confirmed by immunofluorescence microscope and flow cytometry using specific antibodies, as well as embryoid body formation. iPS cells were differentiated into hepatocyte-like cells using a diversity of combinations of growth factors and small molecules targeting the key signaling pathways of the liver development. Hepatic markers alpha fetoprotein and Albumin, the liver cell functions including glycogen synthesis, urea production and inducible cytochrome P450 activity were tested. iPS cells reprogrammed from PBMCs were positive for pluripotency markers NANOG, OCT4, SOX2 and SSEA4, and exhibited the ability of differentiation to three germ layers. The differentiated hepatocyte-like cells from iPS cells displayed the phenotypes and properties of the primary hepatocytes. Albumin and alpha fetoprotein mRNA were detected at day 7. LDL uptake, glycogen storage and urea secretion were observed at day 22. More interestingly, the expression of cytochrome P-450 (CYP) mRNAs in these cells was detected by qRT-PCR and the increased CYP3A enzyme activity was confirmed by the measurement of testosterone 6-beta-hydroxylase activity under the treatment of dexamethasone and rifampicin, which suggests the CYP3A enzyme activity was inducible by drug treatment in these cells. In conclusion, we established an effective and robust strategy for hepatocytes differentiation from iPS cells. These hepatocytes are useful for drug metabolism screening.

T-2069

TO HUMAN PLURIPOTENT STEM CELLS INDUCIBLE OVEREXPRESSION OF RUNX1B BLOCKS THE DEVELOPMENT FROM MESODERM TO EARLY HEMATOPOIESIS

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RUNX1 is absolutely required for the generation of definitive hematopoiesis and the formation of hematopoietic stem cells (HSCs), while it also plays a key role in regulating normal and diseased functions in blood cells. RUNX1b, an isoform of human RUNX1 shows antagonism to RUNX1a in hematopoiesis and leukemia with unknown mechanisms. We applied an inducible expression system to establish RUNX1b-over expression hES cell lines so as to explore the function of RUNX1b in early hematopoiesis. Ectopic over expression of RUNX1b in hESCs inhibits the emergence of human CD34+ hemogenic cells from a very early developing stage before the generation of hemogenic-endothelial cells, thus drastically cut down the production of hamtopoietic stem/progenitor cells (HSPCs). Simultaneously, expression of hematopoietic differentiation-related factors, including RUNX1a, GATA1, GATA2, PU.1 etc, were also negatively regulated. However, over expression of RUNX1b did not affect the development and production hemogenic endothelial cells generated after day-6 coculture, and late HSPCs, proving its blockage happens at very early stage specific before the generation of hemogenic-endothelial cells. Interestingly, this inhibition could be partially rescued by RepSox, an inhibitor of TGF-β signal pathway, indicating a close relationship between RUNX1b and TGF-β signal pathway. Our results suggest an unique inhibitory function of RUNX1b in the development of early definitive hematopoiesis and may benefit to further understanding its biological functions in normal and diseased models.

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T-2071

DIRECTING SKELETAL MYOGENIC PROGENITOR CELL LINEAGE SPECIFICATION WITH CRISPR/CAS9-BASED TRANSCRIPTIONAL ACTIVATORS

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Human pluripotent stem cells (hPSCs) are a promising source for cell therapies, disease modeling, and drug discovery in pathologies of muscular disease. Genetic reprogramming of hPSCs into skeletal muscle cells has been previously demonstrated by exogenous cDNA overexpression of myogenic transcription factors, such as Pax7 and MyoD. Advances in genome engineering technologies have established the CRISPR/Cas9 system as a programmable transcriptional regulator capable of targeted activation of endogenous genes. Nuclease-null dCas9 can be fused to effector domains, such as the transactivation domain VP64, to potently activate genes in their natural chromosomal context. In contrast to ectopic expression of transgenes, activation of endogenous genes can facilitate chromatin remodeling and induction of autonomously maintained gene networks. Targeting endogenous genes can also capture the full complexity of transcript isoforms, mRNA localization, and other effects of non-coding regulatory elements. Here, we use VP64dCas9VP64 to activate endogenous myogenic transcription factor Pax7 in hPSCs to direct differentiation into skeletal muscle progenitors. We hypothesized that activation of Pax7 will induce the myogenic program, as it plays a key role in specification and regulation of muscle progenitor cells. Lentiviral transduction of VP64dCas9VP64 with a Pax7 promoter-targeting gRNA increased transcript levels of multiple Pax7 isoforms, and protein expression was detected 13 days post transduction, indicating robust gene activation. When VP64dCas9VP64 expression is temporally restricted by Cre recombinase-mediated excision from transduced cells, differentiation into myotubes expressing myogenin and myosin heavy chain is observed by day 24 after induction with mesodermal differentiation conditions. Current efforts are assessing the in vivo skeletal muscle regenerative potential of Pax7+ hPSC-derived myogenic progenitor cells by examining engraftment and expansion in immunodeficient mice. These studies introduce a novel method for expansion of myogenic progenitors from hPSCs by deterministic editing of transcriptional regulation with new genome engineering tools, which can enable new possibilities for disease modeling and cell therapy in disorders of skeletal muscle regeneration.

T-2073

ENHANCED NEURAL DIFFERENTIATION ABILITY OF THE HUMAN NAIVE PLURIPOTENT STEM CELLS

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Human pluripotent stem cells, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), show different characteristics compared with mouse ESCs derived from inner cell mass (ICM) of blastocyst stage embryos. It is possible that the difference represents a different developmental stage since the human ESCs/iPSCs closely resemble to mouse epiblast stem cells (EpiSCs) derived from postimplantation embryos. The pluripotent state of mouse EpiSCs is termed primed state to distinguish from naïve state pluripotency of mouse ESCs. Therefore, conventional human pluripotent stem cells also have been regarded as primed state pluripotent stem cells. Although several group have reported the establishment of human naïve pluripotent stem cell, the characteristics vary between reports. Also, the practical advantages of reprogrammed naïve human pluripotent cells remain unclear because the differentiation potential of human naïve cells into specific lineages is not well known. In the present study, we have carried out the conversion of human iPSCs from primed state to naïve state pluripotency by doxycycline (Dox) -inducible reprogramming factors transgenes. The reprogrammed cells were cultured in a medium containing LIF, Dox and cocktail of small molecules. These naïve hiPSCs formed mouse ESC-like dome-shaped colonies and elevated expression of naïve pluripotency-related transcription factors known in mouse ESCs, including ESRRB. Furthermore, we also show that these cells differentiate more readily into neural and glial cells than do conventional hiPSCs. These features may be beneficial for their use in disease modeling and regenerative medicine using hiPSCs.

T-2075

NATURAL KILLER CELL DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

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Natural killer (NK) cells are new candidate sources for immunotherapies in various malignancies. NK cells are a one of innate lymphocytes and show cytotoxicity against tumor cells without prior antigen specific stimulation. More recently, NK cells induction from human pluripotent stem cells (hPSCs), taking the advantage of their unlimited growth potential, has been reported. Although previous studies regarding hPSC-derived NK cells seems impressive and successful, most systems used a bovine and human serum, which might result in the unstable yield and efficiency in the production of HPCs and NK cells. To resolve those problems, we tried to induce functional NK cells from hPSCs under a completely chemically defined condition free from any non-autologous serum or stroma. First, we induced CD34+ and CD45+ hematopoietic progenitors from hPSCs with 85% purity under 2D culture condition. After we collected those cells using magnetic activated cell sorting, we cultured them with NK inducing cytokines. At this point, we selected two media. We compared serum-containing medium and chemically defined medium by evaluating the differentiation efficiency and function of NK cells. On Day 48, the frequency of CD56 positive cells showed no significant differences between two serum-containing medium (79.15 ± 5.30%) and chemically defined medium (80.90 ± 1.27%). In both conditions, NK cells expressed CD56 (NCAM) and specific receptors such as CD161, NKG2D, killer immunoglobulin-like receptors (KIRs), NKG2a (CD94/CD159a heterodimeric inhibitory receptor), NKp44 and NKp46. hPSC-derived NK cells showed the compatible size and morphology to NK cells isolated from peripheral blood NK (PB-NK) cells. PB-NK cells showed 49.65 ± 3.46% of killing activity against K562 target cells, while the killing potential of PSC-derived NK cell's showed killing potential against K562 cells (medium A: 25.4 ± 5.52%, medium B: 23.25 ± 9.26%) which was slightly lower than that of PB-NK cells (49.65 ± 3.46%). In conclusion, we successfully induced functional NK cells from hPSCs under chemically defined condition. They showed compatible phenotype to PB-NK cells in terms of morphology, surface marker and cytotoxicity. They were expected to be applicable not only to immunotherapy but also to model studies of the NKC associating diseases.

PLURIPOTENT STEM CELL: DISEASE MODELING

T-2079

USING IPS CELLS-DERIVED NEURON CELLS AS A MODEL TO TEST THE TOXICITY OF CHINESE HERBS HTL002

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Traditionally, the toxicity of drugs is tested using primary cells. The major obstacle for this testing is the limited availability of the primary cells. Induced pluripotent stem cells(iPSCs) are capable of differentiating to all cell types present in the adult organism, hence the use of iPSC derived tissue specific cells creates a great platform to test the toxicity of drugs. In this report, we tested the neuron toxicity of Chinese herbs HTL002 using iPSC-derived neuron stem cell (NSC). iPSCs were differentiated into NSCs using IxCell Neural Induction System according to the manufacture's protocol. The neural specific marker Nestin was stained and detected by flow cytometry using specific antibody. The toxicity of Chinese herbal was tested at 12 different concentrations (from 0.078125 mg/mL to 25mg/mL). Cell numbers were counted after 48 hour exposure to the herbs using Cell Counting Kit-8 (Beyotime Biotechnology) and absorbance was detected on Tecan M1000 Microplate Reader at 450nm wave length. The matched curve of 50% inhibiting concentration(IC50) is calculated by GraphPad 5.0. More than 95% of the NSCs expressed Nestin during the subculture for 10 generations. After the treatment of herbal HTL002 for 48 hours, the viability of iPS-derived NSCs was significantly decreased ($P < 0.05$) by HTL002 with concentration above 12.5 mg/mL. The number of cells decline more than 51.22% with HTL002 treatment at the concentration of 12.5 mg/mL. The IC50 of HTL002 to iPS-derived NSCs is 12.18 mg/mL after 48 hours of treatment. Nevertheless, Leteprium Potassium, the negative control, showed no toxicity to the NSCs. In conclusion, the results suggest that the iPSC-derived NSCs could be used as an in vitro model for toxicity testing. Chinese herb HTL002 showed significant neuron toxicity at the concentration of 12.5 mg/mL after 48 hours of treatment.

T-2081

THE IPHEMAP DATABASE: AN ATLAS OF PHENOTYPE TO GENOTYPE RELATIONSHIPS OF HUMAN INDUCED PLURIPOTENT STEM CELL MODELS OF NEUROLOGICAL DISEASES, SYSTEMATIC ANALYSIS, RESEARCH PRACTICES AND REPORTING BIAS

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Reprogramming adult human somatic cells into neural cells to model neurological diseases is poised to provide important insights in complex neurological disorders through the demonstration of disease associated phenotypes; however, the abundance of information and the multitude of phenotypes generated from these studies have become difficult to follow and interpret. Here, we examine the current iPSC field practices of neurological disease modeling and report a systematic meta-analysis and field synopsis of phenotype to genotype relationships of 550 distinct neural cell-derived phenotypes extracted by data mining and manual curation of human iPSC experiments performed in 196 patients with neurological diseases and 157 controls. We characterized the resulting relationships between phenotypes and genes into a phenogenetic map by using network biology analysis. We further developed a web-based resource into a publicly available, continuously updated database that catalogs in vitro neural cell disease-phenotypes for data mining: The induced pluripotent stem cell phenogenetic map (http://www.iPhemap.org). Our findings provide new insights into the practice of iPS-derived models and the phenotypes of human-derived neural cells in vitro. Our database provides a tool to catalog patient-derived neural cell phenotypes, to discover disease mechanisms, and to develop novel treatments for human neurological diseases.

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T-2083

IPSC-BASED MODELING OF INTESTINAL COLORECTAL CANCER (CRC)

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We are interested in two major forms of hereditary colorectal cancer (CRC): Familial adenomatous polyposis (FAP) and Lynch syndrome (LS). Directed differentiation of human induced pluripotent stem cells (iPSC) can recreate human intestinal tissue, providing a model to study early carcinogenic events. Samples from FAP and LS patients were reprogrammed into iPSCs. In addition, gene editing was used to create an isogenic platform to compare APC+/+ vs APC+/- cells. Pluripotent cells were first induced to differentiate into definitive endoderm and then pushed towards mid-hindgut and intestinal fate using optimized growth factor cocktails. Monolayer cultures containing positive cells for intestinal markers developed into spheroidal precursors of human intestinal organoids (HIOs). HIOs were collected and matured in 3D matrigel cultures in the presence of intestinal factors and analyzed by morphology, IHC and expression profiles. This platform allowed us to compare FAP, LS and non-diseased lines, describe their molecular profiles and look for new biomarkers of disease. Differences between patients and healthy individuals will help us better understand the etiopathogenesis of the disease and open the door to gene therapy and drug testing.

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T-2085

MODELING MYOTONIC DYSTROPHY TYPE I WITH IPSC DERIVED MUSCLE STEM CELLS

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Myotonic dystrophy type I (DM1) is an inherited muscular dystrophy characterized by a CTG repeat expansion in the 3' untranslated region of the DMPK gene. This expansion results in the expression of a poly(CUG) RNA which sequesters the splicing factor MBLN1 leading to aberrant splicing events, and may be translated into toxic protein species. We derived iPSCs from DM1 patient fibroblasts and differentiated them into skeletal muscle cells. DM1 muscle cells show the expression of toxic poly(CUG) RNA which can be visualized by FISH as RNA foci in the cell nucleus. One therapeutic approach for DM1 is to prevent the expression of the poly(CUG) RNA by blocking transcription of the expanded DMPK gene using a pyrrole-imidazole polyamide (DM1-4) that targets CTG repeats. Preliminary results show reduction of RNA foci in DM1 skeletal muscle cells treated with DM1-4. We have also identified alternative splicing events in iPS-derived DM1 muscle cells compared to unaffected muscle cells and future work will establish whether polyamide treatment will restore these splicing defects.

T-2087

USING HUMAN INDUCED PLURIPOTENT STEM CELLS TO INVESTIGATE THE CONTRIBUTION OF RISK VARIANTS AND AGING TO THE ONSET AND PROGRESSION OF ALZHEIMER'S DISEASE

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Developing therapies for the treatment of Alzheimer's disease (AD) requires an understanding of the mechanisms that cause the disease. Animal models of AD have provided important insights but do not display important AD-related pathologies and have not been useful in modeling the complex genetics associated with "sporadic" AD. Although the majority of AD patients are sporadic, multiple genetic risk variants have been identified, the most powerful and prevalent of which is the E4 variant of Apolipoprotein E (APOE) gene. Compared to individuals with an APOE 3/3 genotype, heterozygosity for the E4 allele increases AD risk by 3 fold, and homozygosity for the E4 allele increases risk up to 12 fold. Amyloid-dependent and -independent mechanisms have been postulated to explain the APOE4 effect, but currently how APOE4 modulates AD disease risk, especially during aging, remains unclear. To that end, we are generating a diverse set of human induced pluripotent stem cell (hiPSC) lines from AD and non-demented control (NDC) patients with no (i.e. APOE 3/3) and two (i.e. APOE 4/4) copies of the E4 allele. We are using these hiPSCs to elucidate the potential genetic, molecular, and cellular mechanisms by which the APOE 4 allele contributes to AD onset and age-related disease progression. By using a novel 3D cortical neuronal culture model and genome-wide expression analysis (RNA-seq), we are identifying unique gene expression profiles that are independently defined by APOE genotype, disease status, and age. Future bioinformatic analysis will reveal candidate genetic,

biochemical, and signaling pathways that will provide more definitive relationships between APOE genotype and AD onset and age-related progression. In the future, we will investigate how modulation of these candidate target genes and pathways regulates the manifestation of AD-related phenotypes. Such future investigations will have significant impact on the design of molecularly targeted therapeutics to treat AD.

T-2089

PATHOLOGICAL MODELING OF NEUROFIBROMATOSIS TYPE 1 DISORDER USING HUMAN PLURIPOTENT STEM CELLS

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Neurofibromatosis type 1 (NF1), also known as von Recklinghausen disease, is an autosomal dominant genetic disorder that affect 1 in 3500 individuals. NF1 is caused by mutation of the tumor repressor neurofibromin 1 gene (NF1) located on chromosome 17. This mutation can be inherited or result from a spontaneous genetic mutation. NF1 is characterized by a wide range of clinical features, from « café-au-lait » spots to bone injuries and neurofibroma. Penetrance of NF1 is complete but the manifestation and severity of the symptoms may vary among individuals within the same family. Sometimes, a second mutation appears, causing the loss of heterozigosity of NF1 and leading to the extinction of neurofibromin, characteristic of additional NF1 lesions such as neurofibromas. Neurofibromas can be cutaneous, sub-cutaneous or plexiform; they are mainly composed of Schwann cells that became invasive. Pluripotent stem cells represent an innovative biological tool to modeling pathologies. To develop a model of NF1, we used a differentiation protocol that leads to generation of Schwann cells precursor derived from human pluripotent stem cells (hPSC). Abnormal proliferation of NF1+/- -hPSC-derived Schwann cell progenitors will be quantified in comparison to control cells and intracellular pathways downstream NF1 will be analyzed. In this context, we will totally knock-out NF1 in precursors of hPSC-derived Schwann cell progenitors and then screen a chemical library to identify compounds with the capacity to restore the normal phenotype.

T-2091

MODELING OF ER-STRESS IN BETA CELLS USING GENOME EDITED PATIENT-DERIVED IPSC CARRYING INSULIN MUTATIONS

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Permanent neonatal diabetes is caused by mutations in genes important for the function of the pancreatic beta cell. Insulin mutations result in the misfolding and endoplasmic reticulum (ER) retention of the insulin protein. Long-term accumulation of aberrant insulin eventually leads to ER-stress, activation of the unfolded protein response pathway and apoptosis of the beta cells. ER-stress is also associated with the progression of T1 and T2 diabetes. To establish a new model for ER-stress induced diabetes, we derived iPSC lines from two Finnish families carrying different heterozygous dominant insulin mutations that result in neonatal diabetes onset at 3-4 months of age. Differentiation efficiency towards the beta cell varies between cell lines with different genetic background. This may obscure the disease phenotype when studying the specific effect of the insulin mutation in beta cells. To overcome this, we generated corrected isogenic cell lines by repairing the insulin gene mutations using CRISPR/Cas9. Corrected cells were differentiated in parallel with mutant cells using an optimized suspension differentiation protocol for 30 days. The resulting islet-like 3D clusters were transplanted under the kidney capsule of immunodeficient mice to complete the beta cell maturation in vivo. We analyzed the islet-like clusters before and three months after transplantation. Increased ER-stress was detected specifically in the INS+ mutant beta cells, with strong BIP (HSPA5) expression progression from a few BIP+INS+ cells before transplantation to 100% after transplantation. Corrected cell lines did not present ER-stress markers at any stage. Insulin-reporter cell lines were generated using CRISPR/Cas9 from mutant and corrected cell lines, enabling INS+ cells FACS-sorting followed by RNA-seq. We also conducted single cell RNA-seq of islet-like clusters before transplantation to study the transcriptional changes at the cell level. We envision this model will provide new insights into the molecular mechanisms behind ER-stress based demise of the beta cells and serve as a drug-screening platform for novel antidiabetic agents.

T-2093

INVESTIGATING DOXORUBICIN INDUCED CARDIOTOXICITY IN HUMAN PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

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Doxorubicin is a highly effective chemotherapeutic drug but causes cardiotoxicity in up to 26% of patients. Despite extensive study, the mechanisms of this adverse drug reaction are unknown. The objective of this study was to investigate the molecular mechanisms of doxorubicin-induced cardiotoxicity (DIC) using human pluripotent stem cell (PSC) derived cardiomyocytes (CM). We used both human embryonic stem (hES) cells and patient-specific induced pluripotent stem cells (iPSCs) to study the molecular mechanisms of DIC. iPSCs were generated from PBMCs from doxorubicin-treated patients using the Sendai virus method. PSCs were differentiated to cardiomyocytes using inhibitors of Wnt and GSK3 in insulin-deficient media. Doxorubicin toxicity was assessed using various cellular and electrophysiological assays, cell viability, reactive oxygen species (ROS) production, double stranded DNA breaks and optical mapping. Doxorubicin caused dose-dependent effects on increasing apoptosis and necrosis, generation of reactive oxygen species, mitochondrial dysfunction and intracellular calcium concentration. We studied the effect of TOB2B, a gene implicated in DIC from mouse studies, using CRISPR-Cas9-mediated disruption of the gene. Deletion of TOP2B reduced doxorubicin-induced double stranded DNA breaks and cell death, proving the important role of TOP2B in DIC in human cardiomyocytes. Using optical mapping, we showed that doxorubicin caused a decreased beating rate and delayed action potential upstroke, increased calcium transient duration and action potential duration with the emergence of delayed after depolarizations. Our data establish a PSC-derived CM model of DIC. This model could be used to predict sensitivity to this ADR prior to the administration of doxorubicin.

T-2095

LOSS OF THE TRANSCRIPTIONAL REPRESSOR MUSCLEBLIND-LIKE 3 (MBNL3) CAUSE CANCER STEM CELLS TO SWITCH TO AN EMBRYONIC ALTERNATIVE SPLICING PATTERN IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Since the establishment of human embryonic stem cell (hESC) lines and the discovery of stem cell characteristic induction in somatic cells by transcription factors OCT3/4, SOX2, C-MYC, and KLF4, human pluripotent stem cell research has provided key insights into fundamental facets of human developmental biology. Formative research suggests that a hESC-specific alternative splicing gene regulatory network, which is repressed by Muscleblind-like (MBNL) RNA binding proteins, is involved in cell reprogramming. By extensive leukemia stem cell whole transcriptome RNA-sequencing, lentiviral overexpression and knockdown we discovered that decreased expression of MBNL3, a repressor of an embryonic alternative splicing program and reprogramming, activated a pluripotency network and increased expression of a pro-survival isoform of CD44v3, which is more commonly expressed in hESCs. This resulted in malignant reprogramming of progenitors in blast crisis CML endowing them with unbridled survival and self-renewal capacity. This is the first description of MBNL3 downregulation as a mechanism of reversion to an embryonic alternative splicing program, which elicits malignant progenitor reprogramming of progenitors into self-renewing leukemia stem cells (Holm et al., 2016 PNAS). Since then, we have identified this phenomenon in acute lymphoblastic leukemia (ALL), thus indicating the importance of MBNL3 in multiple forms of leukemia. In an effort to elucidate the particular role of MBNL3 in human leukemia stem cell generation we have identified that the main proteins of the TGFβ family, SMADs, play an important role in the regulation of MBNL3 in ALL. By RT-qPCR we found an upregulation of the inhibitory SMADs, SMAD6 and SMAD 7 in ALL in comparison to

cord blood control samples. On the contrary SMAD 2 and SMAD3 were downregulated. Upon MBNL3 lentiviral knockdown in cord blood samples an upregulation of SMAD6 and SMAD7 where identified. Additionally, we performed chromatin immunoprecipitation coupled with PCR (ChIP-PCR) in order to identify important binding partners. We found that SMAD6 binds to the promoter of pluripotency markers such as OCT4, SOX2 and NANOG, indicating a relationship between MBNL3 and SMAD6 in its regulation of an embryonic alternative splicing program in acute lymphoblastic leukemia.

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T-2097

DEVELOPMENT OF AN IPSC MODEL OF MLL-AF4+ INFANT B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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B-cell acute lymphoblastic leukemia (B-ALL) is the most common cancer in childhood. Despite overall survival rates of over 85%, high-risk subtypes such as infant B-ALL still associate with poor clinical outcome and response to therapy. Infant B-ALL sees the highest frequency of rearrangements involving the KMT2A/MLL gene, with the t(4;11)(q21;q23) MLL-AF4 translocation found in approximately 50% of cases correlating with a dismal prognosis. Strong evidence suggests that this main leukemogenic event targets a developmentally-restricted population and is completed during prenatal life. Further understanding of the biology of this disease has been hampered by a lack of models that fully recapitulate the human disease, warranting the development of new bona-fide models. Induced pluripotent stem cell (iPSC) modeling offers the unique opportunity to recapitulate the developmental progression of malignancies and could enable the modeling of the in utero haematopoietic defects of infant B-ALL. To this end I have been generating iPSCs from cell lines and primary patient samples of B-ALL using several reprogramming methods. Derivative iPSCs were obtained at an extremely low efficiency (0.0001%). The iPSC lines were assessed for the full reactivation of pluripotency by several assays including spontaneous differentiation of iPSC-derived embryoid bodies, in vivo teratoma formation and RNA sequencing. B-ALL iPSCs are currently being differentiated to the haematopoietic lineage to demonstrate their ability to generate the lineage of interest. All iPSC lines were screened for the presence of the translocation by FISH analysis but repeatedly failed to present the rearrangement of the parental population. One partially-reprogrammed iPSC line derived from a t(4;11)+ cell line was positive for the translocation albeit in abnormal copy numbers, suggesting a negative selective pressure against the generation of MLL-AF4+ iPSCs. Current efforts are focused on investigating the mechanisms underlying the low efficiency of leukemia reprogramming. The establishment of an iPSC model of MLL-AF4+ B-ALL could allow an unprecedented insight into the early stages of MLL-AF4 leukemogenesis, with the potential to improve the treatment and survival rate of this high-risk subgroup.

T-2099

DERIVE THE INDUCED PLURIPOTENT STEM CELLS FROM WOMEN WITH POLYCYSTIC OVARY SYNDROME TO OVARIAN GRANULOSA-LIKE CELLS

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The inherited trait of polycystic ovarian syndrome (PCOS) is still controversial. Chronic anovulation and disturbed ovarian folliculogenesis are main characteristics in women with PCOS. We established the PCOS-specific induced pluripotent stem cells (iPSCs) and firstly differentiate them into ovarian granulosa-like cells. We further compared the difference of differentiated granulosa-like cells derived from iPSCs between women with and without PCOS. Cells originating from skin fibroblasts of two women each with and without PCOS were reprogrammed by using origin of replication/ Ebstein-Barr Nuclear Antigen-1 (ori/EBNA-1)-based episomal vectors carrying defined factors. The resulting iPSCs were then differentiated into ovarian granulosa-like cells using multistep approaches comprising in vitro treatments with cocktails of growth factors. The establishment of pluripotent characteristics and expression of differentiation and granulosa cell markers were analyzed by real-time PCR, Western blotting, array cGH, immunofluorescence, flow cytometry, and in vivo and in vitro differentiation assays. ELISA, RT-PCR and aromatase activity assay were used to analyze differentiated granulosa cell function. Gene expression analyses revealed the granulosa cell specific markers including AMH, type 2 AMH receptor (AMHR2), FSH receptor, LH receptor, estrogen synthetase cytochrome P450 19A1 (CYP19A1), and forkhead transcription factor [FOXL2] all significantly expressed in differentiated granulosa-like cells derived from both PCOS and non-PCOS iPSCs when compared to undifferentiated iPSCs. After FSH stimulation, the expression of AMHR2, CYP19A1, FOXL2, and FSHR in granulosa-like cells differentiated after 12 days was not significantly different between PCOS iPSCs and non-PCOS iPSCs. While the aromatase activity of the differentiated granulosa-like

cells from PCOS iPSCs was significantly higher than those from non-PCOS iPSCs. Our study firstly reported a successful differentiated granulosa-cell differentiated from iPSC of women with PCOS to investigate the disturbed ovarian folliculogenesis and steroidogenesis in women with PCOS.

Funding Source: This study was supported by grants MOST 102-2321-B002-093-MY3 from Ministry of Science and Technology of Taiwan.

T-2101

INDUCED PLURIPOTENT STEM CELL DISEASE MODELING OF LMNA-ASSOCIATED CARDIOMYOPATHY REVEALS PERTURBATIONS IN EMBRYOID BODY FORMATION

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Lamin A/C are intermediate filament proteins encoded by the LMNA gene that construct the nuclear lamina of a cell and implicated in processes such as gene expression and cell differentiation. Consequently, mutations in LMNA have detrimental effects in cell functions which results in diseases known as laminopathies. To elucidate the role of LMNA mutations in laminopathies, we are developing patient-derived induced pluripotent stem cells (iPSC) to model cardiac disease in a family with dilated cardiomyopathy and arrhythmia due to a heterozygous splice site mutation. This mutation is predicted to result in exon skipping and subsequently Lamin A/C haploinsufficiency. Here, we report our observations to test the hypothesis that Lamin A/C plays an important role in iPSC differentiation. Primary adult dermal fibroblasts from an affected individual (patient) and an unrelated individual (control) were reprogrammed into iPSC. After multiple passages, patient and control iPSC clones were assessed for: pluripotency by gene expression and immunostaining of pluripotency markers; differentiation capability by directed differentiation to the three germ layers and spontaneous formation of embryoid bodies (EB); and chromosomal stability by karyotype. Our results showed that both patient and control iPSC were pluripotent based on presence of pluripotent markers and expression of Yamanaka factors by real-time PCR. In addition, both patient and control iPSC differentiated to cells from the three germ layers based on positive staining and real-time PCR for lineage-specific markers. However, despite these findings along with normal karyotypes for both lines, we found that patient iPSC generated deformed EBs. Prolonged culture of patient EBs produced lineage-specific cells; however, these EBs possessed qualitative defects in formation, shape, and number. These observations of LMNA-associated defects in EB formation suggest possible roles of Lamin A/C in apoptosis, cell kinetics, and/or pattern formation. Further studies using additional patient IPSC and quantitative studies on EB formation are anticipated to elucidate on these observations and how specific mutations contribute to the pathology of LMNA-associated cardiomyopathy.

Funding Source: This work was supported by grant 1R01HL129008 from the NIH National Heart, Lung, and Blood Institute.

T-2103

IPSC-DERIVED NEURAL CELLS FROM PATIENT WITH GM2 GANGLIOSIDOSES (TAY-SACHS DISEASE) FOR PATHOLOGICAL STUDIES

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GM2 gangliosidosis belongs to a group of inherited lysosomal storage disorders caused by deficiency of β-hexosaminidase, which leads to excessive accumulation of GM2 ganglioside and related glycolipids in the lysosomes of mainly neuronal cells of central nervous system. There are two isoenzymes of β-hexosaminidase, A and B (HEXA and HEXB). Mutations in the HEXA gene cause Tay-Sachs disease, whereas mutations in both HEXA and HEXB genes cause Sandhoff disease (GM2-gangliosidosis variant 0). Defect in degradation of GM2 gangiosid can be also caused by mutation in GM2 activator protein gene, and the disease is known as GM2-gangliosidosis variant AB. Individuals with GM2 gangliosidosis experience progressive neurological diseases including motor skills lose, progressive weakness and hypotonia, decreased responsiveness, vision deterioration, and seizures. GM2-gangliosidosis is a rare disease and access to patient's samples is very limited, thus studies focused on understanding its pathogenesis and exploring potential treatment procedures mostly rely on experiments performed on animal models. Relatively new approach, induced pluripotent stem cells (iPSC) technology, also offers similar alternative tools, which can be applied to study inherited metabolic disorders including GM2-gangliosidosis. We used GM2-gangliosidosis patient's blood cells for generation of disease-specific iPSC which expressed typical markers of pluripotency and can be differentiated to all three germ layers. Since the understanding of neurodegenerative involvement of the disease is incomplete, we decided to differentiate iPSC into neuronal and glial cells for pathological studies.

Increased accumulation of GM2 ganglioside has been demonstrated in a mixture of neural cells by ESI-MS/MS analysis as well as increased accumulation of lysosomal marker LAMP1 in the same cells by western blot analysis. Immunofluorescent analysis confirmed presence of β-Tubulin III and MAP2 positive neurons, GFAP positive astrocytes and CNPase positive oligodendrocytes. Increase amount of LAMP1 protein was confirmed in all cell types compared to control and analysis by electron microscopy showed lysosomal abnormalities mainly in iPSC-glial of the patients.

Funding Source: This work was supported by project RVO-VFN64165; Grant GAUK 56214 and AZV 15-33297A.

T-2105

AN IPSC MODEL OF THE NEUROMUSCULAR JUNCTION IN ALS

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Amyotrophiclateralsclerosis(ALS)isaneurodegenerative disease that affects primarily upper and lower motor neurons (MNs). A small percentage (10%) of cases are familial (fALS), but most cases are sporadic (sALS). The neurodegeneration of MNs occurring in ALS includes not only MNs but also non-cell autonomous effects of the cells in the surrounding micro-environment. One of the earliest pre-symptomatic events during ALS pathology is the loss of structure and innervation at the level of the neuromuscular junction (NMJ), both in patients and in mouse models of the disease. This would suggest a possible role of the skeletal muscle itself in the pathology of ALS. However, while it is generally accepted that astrocytes and microglia play a role in ALS, the contribution of muscle to NMJ degradation is controversial. We hypothesize that the skeletal muscle compartment plays a key role in modulating MN survival and overall ALS pathology. To answer this, we have developed an in vitro model of the human NMJ, in which both neuronal and muscular components of the NMJ are derived from human induced pluripotent stem cells (iPSCs). These NMJs show co-localization of presynaptic and postsynaptic NMJ markers and demonstrate physiological activity, with contraction of the skeletal muscle component upon optogenetic neuronal stimulation. This model has the advantage a patient-specific approach, in that IPSCs can be used to mirror the pathophysiology of different disease subgroups, such as sALS, for which animal models do currently not exist. We have used this IPSC-derived in vitro model to understand the timeline of events that leads to the degradation of the NMJ during ALS, using fALS lines for SOD1A4V and SOD1G85R, TDP-43G298S, and C9orf72 as well as sporadic cases. When available, isogenic corrections of point mutations (or isogenic lines with pathologic and non-pathologic hexanucleotide repeats) where used as controls, otherwise age-matched controls were used. We used a combinatorial, 2x2 approach to identify the distinct contributions of the motor neuron and muscle to NMJ degeneration, and establish when motor neuron terminal retraction and NMJ degeneration occur relative to disease processes in more proximal MN compartments, like the axon and cell body.

T-2107

RECAPITULATION OF DISEASE-PHENOTYPE USING PATIENT-SPECIFIC IPSCS OF OSTEOGENESIS IMPERFECTA

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Osteogenesis imperfecta (OI) is one of the congenital bone diseases and is caused by mutations affecting the structural and functional property of type I collagen. Most of the patients have either COLA1 or COL1A2 mutations, but some patients have mutations in other genes involved in collagen maturation. OI is characterized by increased bone fragility, low bone mass, short stature and other connective tissue manifestations, and clinically classified into four types. Most severe form of OI (Sillence type Ⅱ) is perinatal lethal due to respiratory problems. Bisphosphonates have been used as a therapeutic drug for this disease, but the effects are limited. To develop novel drugs, in vitro assay system suitable for high throughput screening will be desirable, although it is hardly possible by using patient-derived somatic cells because of their limited growth. Application of patient-derived induced pluripotent cells (iPSCs) can overcome this problem. We have established iPSCs from fibroblasts of eight patients of OI, all of which are classified Sillence type III (severe type). Exome analyses identified COL1A1 and COL1A2 mutations in six and two patients, respectively. Using the osteogenic induction method established in our laboratory, iPSCs of these patients were induced into osteoblasts and further osteocytes, and the property of

osteogenic cells such as the production of collagen and mineralization were evaluated and compared with those of standard iPSCs. As a result, we have successfully recapitulated abnormalities in osteogenic property in these cells, suggesting that our in vitro system will be useful tool to identify new drugs for OI.

T-2109

SINGLE-CELL ANALYSIS OF NEURAL STEM CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS CARRYING LRRK2 MUTATION GLY2019SER

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Parkinson's disease (PD) is a neurodegenerative disorder caused by the loss of dopaminergic neurons in the substantia nigra. It is characterized by tremors, rigidity, bradykinesia, akinesia and cognitive impairment. Even though PD is considered an idiopathic disease, at least 10 percent of cases are hereditary. A common genetic mutation found in familial PD was identified in the LRRK2 gene. LRRK2 has known kinase and GTPase activity, with functions linked to autophagy and mitochondrial function. However, it is still unknown how this mutation causes PD. To further investigate the LRRK2 mutation, induced pluripotent stem cells (iPSC) were generated from the fibroblasts of a PD patient with the LRRK2 mutation, Gly2019Ser. To explore the expression profile of the iPSC-lrrk2, Single Cell RNA-sequencing (scRNA-seq) was performed on neural stem cells (NSC). Unlike bulk RNA-seq, scRNA-seq allows us to measure the expression profile of individual cells, study cell heterogeneity, and identify highly variable genes across the population. In this study, NSCs were generated using a chemically defined media supplemented with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). After 1 week of culture, neurospheres were dissociated into single cells. Using the microfluidic device Fluidigm C1, RNA was extracted from 68 individual cells and cDNA libraries were generated using Illumina Nextera XT. Sequencing was performed using the Illumina HiSeq 3000 with 100 bp paired-end sequencing providing us with an average of 4 million reads per sample. The reads were pseudoaligned and quantified using Kallisto. Heterogeneity of the cell population was analyzed by generating PCA, t-SNE, and heatmap plots, which revealed 4 distinct populations. Preliminary results of DAVID analysis show a significant enrichment for PD and oxidative stress related genes. Further analysis of various subpopulations of undifferentiated neural stem cells and differentiated dopaminergic neurons will be presented.

T-2111

MODELING PEDIATRIC GLIOMAS OF THE THALAMUS BEARING HISTONE MUTATIONS

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Pediatric high-grade gliomas are malignant brain tumors arising in children that are associated with very poor clinical outcomes. Recent efforts to profile the genetic alterations found in these tumors have revealed frequent mutations in histone H3 genes, including lysine-to-methionine "H3K27M" mutations in 80% of diffuse intrinsic pontine gliomas (DIPGs) and other subcortical gliomas. Tumors with H3K27M mutations, including DIPGs, possess somatic alterations in a variety of growth factor signaling pathways as well. Such co-alterations include activating mutations in the fibroblast growth factor (FGF) receptor FGFR1 in tumors that arise preferentially in the thalamus. The roles of these mutations in tumorigenesis remain unclear; moreover, their potential values as therapeutic targets have yet to be fully explored. Here, we aim to establish a model of pediatric thalamic gliomas harboring FGFR1 and H3K27M mutations. To date, our findings indicate that H3K27M reduces overall H3K27 trimethylation in neural precursor cells (NPCs) expressing thalamic progenitor-associated genes along with activated FGFR1. We have found that both of these mutations drive a variety of cancer-associated phenotypes in these NPCs in vitro and that NPCs bearing the mutations can be transplanted into the mouse brainstem to model the disease in vivo. We have also found that activated FGFR1 and H3K27M increase expression of genes associated with early neuroectodermal cells but not highly expressed in NPCs, including LIN28A and LIN28B, and that multiple mechanisms may underlie their upregulation. Finally, we report that targeting FGFR activity with a small-molecule inhibitor depletes wild-type NPCs and NPCs bearing the mutant receptor at similar doses, thus indicating limited efficacy and potential toxicity of this therapeutic strategy. Further efforts are underway to thoroughly characterize this model in vitro and in vivo via cell grafting, with the goal of providing novel insights into the biology and potential treatment of these refractory pediatric brain tumors.

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T-2113

EPIGENETIC CHANGES IN NEURAL CELLS DERIVED FROM CD34+ CELLS FROM PATIENTS WITH PRIMARY LATERAL SCLEROSIS

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Primary lateral sclerosis (PLS) is a neuromuscular disorder characterized by progressive weakness of voluntary muscles caused by the chronic degeneration of upper motor neurons in the central nervous system. The pathogenesis of PLS is largely unknown and its study is hindered by the lack of access of patient specific neurons to construct in vitro disease models. Recent developments in the field of cell transformation make it possible to derive neural cells directly or through induced pluripotent stem cells (iPSCs) by transfecting other somatic cells with Yamanaka factors. Here we directly generated neural progenitors and induced pluripotent stem cells from PLS patients to study the epigenetic changes of the cells and their possible roles in regulating neural development. Neural progenitor cells and iPSCs were derived by transfecting enriched CD34+ cells from blood samples from PLS patients with Sendai virus containing Yamanaka factors (Oct4, Sox2, C-Myc and Klf4). The neural progenitor cells were further differentiated to neurons using differentiation medium. The general DNA methylation of the cells were measured using a LINE-1 methylation detection kit. The neuronal development was studied by using the 3D cultures of the iPSC-derived neural spheres. Neural cells differentiated from PLS patients showed significantly lower level of DNA methylation compared to their counterparts derived from normal donors. Furthermore, the phenotype of 3D neural spheres derived from PLS patents also showed difference compared to the ones from normal donors. These results indicate that directly derived neural cells from CD34 cells from PLS patients may have different epigenetic status compared to the normal controls and which may affect the neural development.

REPROGRAMMING

T-2115

MICROPATTERNING OF REPROGRAMMING CULTURES TO TRACK AND CONTROL NUCLEAR PROPERTIES FOR THE PRODUCTION OF HIGH-QUALITY IPSCS

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Molecular mechanisms of reprogramming terminally differentiated cells to a pluripotent state still are poorly understood. As a result, standard reprogramming techniques are noisy and inefficient. To address these shortcomings, we have developed a novel platform that allows for the dynamic tracking of subpopulations in a longitudinal manner across extended periods of time. This platform is a simple approach that combines live-cell microscopy with surface-modified multiwell plates that separates thousands of cell populations. With this we are able to both watch and physically constrain cells into discrete islands during reprogramming. By watching subpopulations in real time we are able to distinguish intermediate states that either contribute or are detrimental to complete reprogramming. Furthermore by controlling the island geometry at the microscale, we are able to selectively activate mechanotransduction pathways (e.g., YAP/Taz) to promote transition through the endothelial-mesenchymal transition during the reprogramming process. Direct manipulation of the YAP/Taz pathway via lentiviral overexpression also promoted reprogramming. Overall, this simple platform allows us to expand our understanding of intermediate cell states and increasing the overall efficiency of the reprogramming process.

T-2117

ENHANCED CONVERSION OF ADULT HUMAN FIBROBLASTS INTO INDUCED NEURAL PRECURSOR CELLS USING CHEMICALLY MODIFIED MRNA

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Direct reprogramming offers a unique approach by which to generate mature neural lineages for the study and treatment of neurological diseases and

neurodevelopmental disorders. We have developed an efficient system for directly generating neural stem/precursor cells (iNPs) from adult human fibroblasts by transient ectopic expression of the neural-promoting transcription factors, SOX2 and PAX6. This was achieved using plasmid cDNA which, while desirable in that it reduces potential genomic integration of ectopic factors, results in relatively poor transfection efficiency compared to traditional viral transduction. To advance our direct reprogramming technology, we optimized a chemically modified mRNA gene delivery system (SNIM®) for direct iNP reprogramming. SNIM® RNA has the benefit of being extremely stable and non-immunogenic, allowing us to co-transfect adult human fibroblasts with our reprograming factors SOX2 and PAX6 with an efficiency of >75%, higher than the 10-20% transfection efficiency obtained with plasmids. Cell viability was >70% post-transfection, significantly greater than 20-40% viability obtained with plasmid transfection. Co-transfection of SOX2 and PAX6 using SNIM® RNA also increases the rate of iNP reprogramming to 14 - 28 days compared to ~45-65 days required using plasmid transfection. The expression of a wide range of neural positional genes were observed through qPCR, and differentiation of SNIM® RNA-derived iNPs generated a mixed neuronal population of a predominantly GABAergic and glutamatergic phenotype. This represent the first time a chemically modified mRNA approach has been used to directly reprogram adult human fibroblasts to iNPs, and provides a rapid system by which to generate human neurons for both research and clinical application.

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T-2119

ENHANCED SINGLE CELL VIABILITY OF HUMAN IPSC USING 30KC6 GENE

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Since the finding of human induced pluripotent stem cell (hiPS cell), several studies have been made on hiPS cell. Attention has focused on the use of hiPS cell in regenerative medicine because of their pluripotency. Also, hiPS cell could avoid ethical issues that may arise in human embryonic stem cell. However, the culture of hiPS cell is time consuming and labor intensive. Unlike mouse iPS cell, hiPS cell is required for colony transfer because single cell occurs apoptosis easily. In this study, we applied 30Kc6 gene which is derived from Bombyx mori, and known as anti-apoptosis property on hiPS cell. 30Kc6 gene was expressed in hiPS cell, and the hiPS-30Kc6 cell expressed pluripotency markers (Oct4, Tra-1-60, and alkaline phosphate). When the cells were induced apoptosis by staurosporine, hiPS-30Kc6 cell was showed the 1.4 times less annexin V expression comparing with normal hES cell or hiPS cell. Moreover, the viability of enzymatic single cell dissociation was 3 times increased in hiPS-30Kc6 cell by comparing with normal hiPS cell. It means that the introduction of 30Kc6 seems to affect hiPS cell by inhibiting of apoptosis. Therefore, the technology using 30Kc6 is anticipate to be used on stem cell engineering.

T-2121

DIRECT IN VIVO GLIA-TO-NEURON CONVERSION IN THE POSTNATAL MOUSE CEREBRAL CORTEX

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We previously showed that forced expression of neurogenic transcription factors can reprogram cortical glia into immature neurons in vivo in the injured adult brain. Importantly, such factor-induced cell fate switch only occurred in the context of brain lesion, which is known to elicit local glial proliferation. To clarify the influence of the milieu on the reprogramming process we tested here whether proliferative glia can be lineage converted in vivo in the intact postnatal mouse cortex. Glial cells proliferate locally in the early postnatal cortex. To target these cells for lineage conversion we transduced proliferating glia with retroviruses encoding for neurogenic transcription factors. Unlike earlier reported in vitro, overexpression of the transcription factors Ascl1 and Neurog2 alone was insufficient to induce the generation of neurons from postnatal cortical glia in vivo. This suggests that additional factors may be required to induce glia-to-neuron conversion in vivo. Thus, we firstly tested whether co-expression of Sox2 can facilitate reprogramming induced by Ascl1. Consistent with the previously observed synergism

between Sox2 and Ascl1 on lineage reprogramming of adult cells, we found that combined expression of these two transcription factors results in the lineage conversion of proliferative glia into doublecortin-positive neurons in the postnatal cortex in vivo in the absence of a lesion. Likewise, we show that additional factors can enhance Neurog2-mediated glia-to-neuron conversion in the non-injured postnatal cortex as illustrated by expression of immature and mature neuronal markers and the acquisition of a highly elaborated neuronal morphology. Patch-clamp recordings further showed that these induced neurons acquired voltage-dependent sodium and potassium currents resulting in the ability of repetitive action potential firing. Our data show that glia-to-neuron reprogramming can be elicited in the absence of prior injury, but may require ongoing proliferation.

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T-2123

FUNCTIONAL MATURATION OF DIRECT CONVERTED HEPATOCYTES

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The field of direct reprogramming is currently being investigated very rapidly. Unlike induced pluripotent stem cells, this technique enables direct differentiation into the other cell types such as neurons, cardiomyocytes, insulin-producing cells, and hepatocytes without going through the pluripotent stage. Direct reprogrammed induced hepatocyte-like cells (iHeps) have potential as cell therapy for a variety of liver diseases and are also applicable to drug screening studies. To achieve these goals, however, it has to be possible to produce mature hepatocytes in vitro. Recent studies show that the direct reprogramming technique can differentiate into iHeps in vitro, but the differentiation into functionally mature hepatocytes for this purpose is still difficult. This study produces mature hepatocytes from hepatocyte-like cells induced by direct reprogramming by treatment with DMSO. Using two transcription factors HNF4α and Foxa3, we produce direct reprogrammed from mouse embryonic fibroblasts (MEFs) with up to 80% efficiency within 2 weeks. Direct reprogrammed cells indicate typical epithelial morphology, obtain hepatocyte functions and express hepatic genes. After maturation, iHeps showed features of maturation which more typical hepatic morphology, more express hepatic genes, and acquired hepatic function over time. Our data provides a novel strategy to functionally mature directly induced hepatocyte-like cells and these maturated iHep applicable for the purpose of cell therapy, regenerative medicine and live engineering.

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T-2125

A CONSERVED APPROACH TO NON-INTEGRATING REPROGRAMMING OF MAMMALIAN CELLS

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The derivation of induced pluripotent stem cell (iPSC) lines from a wide range of mammalian species provides a valuable resource for comparative developmental studies. Routine reprogramming of human patient cells is being used to capture the genetic diversity of individuals to generate new cell models that can recapitulate developmental and disease processes in a dish. This strategy can be extended across genera to generate putative iPSCs from a wide range of mammalian species, using a common set of core reagents to select for pluripotent cells derived under conditions similar to those used for human iPSCs. The conservation of identity of key transcription factors is sufficient such that transduction with vectors expressing the human Oct4, Sox2, Klf4 and c-Myc cDNAs can reprogram somatic cells from a wide range of mammals without the need to clone species specific genes. A non-integrating Sendai RNA viral vector system was used to effectively reprogram fibroblasts from a range of felid species (Domestic and Asian Leopard Cat, Cheetah) and Non Human Primate species (Rhesus Macaque and Gorilla) into stable, putative iPSC lines. While species-specific differences may exist in growth factor dependence during embryonic development in vivo, by using existing media systems that support conserved signaling pathways, putative iPSC lines were selected for that survived in vitro in human iPSC culture conditions. Of practical utility, these putative iPSC lines grow rapidly and can be cultured well beyond the point at which the parental fibroblast lines undergo senescence. The majority of putative iPSC lines derived

by this non-integrating method maintain a normal diploid karyotype determined by Giemsa banding analysis. Standard in vitro and in vivo assays are underway to test the differentiation capabilities of these cell lines. Non-integrating, Sendai viral vector-based reprogramming provides a standardized and reproducible approach to generating viable animal cell models free of DNA-artifacts that complement human genome resource and cell banking efforts.

T-2127

HIGH-RESOLUTION DISSECTION OF DETERMINISTIC SOMATIC CELL REPROGRAMMING TO NAÏVE GROUND STATE PLURIPOTENCY

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The ability to reprogram somatic cells into iPSCs with four transcription factors (classically Oct4, Sox2, Klf4 and cMyc) has provoked immense interest over the last decade. Different studies sought to define the molecular dynamics of the process. Despite important progress, major questions regarding comprehending the dynamics of epigenetic reprogramming at high resolution and in correctly reprogrammed iPSCs, remain vaguely defined. This gap in understanding results from the inefficiency of conventional reprogramming methods coupled with the difficulty of prospectively isolating the rare cells that eventually correctly reprogram into iPSCs. Here we characterize cell fate conversion from fibroblast to induced pluripotent stem cell (iPSC) by a highly efficient murine deterministic reprogramming system with controlled reduced expression of Mbd3-Gatad2a/NuRD complex. This characterization was done in a single-day resolution, and includes inspection of gene expression, chromatin modifications, TF binding, DNA accessibility and methylation. This integrative analysis identified two transcriptional modules that dominate successful reprogramming. One consists of genes whose transcription is regulated by switching epigenetic modifications in their promoters (abbreviated as ESPGs) and the second consists of genes with a constitutively active chromatin state, but dynamic expression pattern (abbreviated as CAPGs). ESPGs are mainly regulated by OSK, rather than Myc, and are enriched for cell fate determinants and pluripotency factors. CAPGs are regulated mainly by c-Myc, and they are enriched for cell biosynesthetic regulatory functions. We used ERPG group to study the identity and temporal occurrence of activating and repressive epigenetic switching during reprogramming. Moreover, CAPGs are adequately regulated by multiple ways: 1) selective reduction in CAPG mRNA degradation rate 2) change in tRNA codon usage 3) Myc activity, endogenous or exogenous, dominates CAPG expression. In summary, our study underscores the benefits of conducting unbiased high-resolution mapping of epigenetic changes on somatic cells that are deterministically committed to undergo successful reprogramming

T-2129

REPROGRAMMING OF HUMAN PERIODONTAL LIGAMENT FIBROBLASTS TO INDUCED PLURIPOTENT STEM CELLS UNDER XENO-FREE CONDITIONS

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Over recent years, the ability to generate human induced pluripotent stem (iPS) cells derived from somatic cells provides tremendous promises for regenerative medicine and its use has widely increased. Choice of reprogramming tools was important for the safety concerns remain and the increasing efficiency of reprogramming process, transduction. Non-integrating techniques as like mRNA or protein-based reprogramming protocols are available for the production of genetically stable iPS cells. In 2015, Poleganov et al reported mRNA based reprogramming have displayed unique and independent disadvantages, such as being inconsistent and inefficient, requiring expensive and complicated protocols with long derivation timelines, or intensive monitoring of established iPS cell lines for the elimination of persistently replicating delivery vectors. Therefore there is an imminent need for a simple, reproducible, and more economical method to generate integration-free clinical grade iPS cells. In this study, we were isolated human periodontal ligament fibroblasts (PDL) to primary cultures from dental patients and they can be used as the cell source for iPS generation under xeno-free conditions. PDL reprogramming was produced by non-integration methods using the combination of reprogramming mRNAs [OCT4, SOX2, KLF4, cMYC, NANOG and LIN28 (OSKMNL)] with evasion mRNAs [E3, K3 and B18R (EKB)] (StemRNA-NM Kit, Stemgent, USA) during 4 days. Reprogrammed colonies were appeared after 8 days of transduction and identified using TRA-1-81 antibody (StainAlive™, Stemgent, USA) by immunocytochemistry (ICC) of live cells in culture that are able to be isolated from the primary culture between Day 10-14 into individual wells. Established iPS cells were expanded on iMatrix-511 in NutriStem XF/FF and stained for pluripotency associated genes. Overall, this presented results demonstrate that human iPS cells can be generated and maintained under xeno-free conditions and may provide a path to good manufacturing practice (GMP) applicability that should

facilitate the clinical translation of patient-specific or disease-specific iPS cells therapies.

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T-2131

HOMOGENEOUS GENERATION OF IDA NEURONS WITH HIGH SIMILARITY TO BONA FIDE DA NEURONS USING A DRUG INDUCIBLE SYSTEM

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Recent work generating induced dopaminergic (iDA) neurons using direct lineage reprogramming potentially provides a novel platform for the study and treatment Parkinson's disease (PD). However, one of the most important issues for iDA-based applications is the degree to which iDA neurons resemble the molecular and functional properties of their endogenous DA neuron counterparts. Here we report that the homogeneity of the reprogramming gene expression system is critical for the generation of iDA neuron cultures that are highly similar to endogenous DA neurons. We employed an inducible system that carries iDA-inducing factors as defined transgenes for direct lineage reprogramming to iDA neurons. This system circumvents the need for viral transduction, enabling a more efficient and reproducible reprogramming process for the generation of genetically homogenous iDA neurons. We showed that this inducible system generates iDA neurons with high similarity to their bona fide in vivo counterparts in comparison to direct infection methods. Thus, our results suggest that homogenous expression of exogenous genes in direct lineage reprogramming is critical for the generation of high quality iDA neuron cultures, making such culture systems a valuable resource for iDA-based drug screening and, ultimately, potential therapeutic intervention in PD.

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TECHNOLOGIES FOR STEM CELL RESEARCH

T-2133

CULTURE ENGINEERED MESENCHYMAL STROMAL CELLS (MSCS) WITH ENHANCED ANTI-INFLAMMATORY AND ANTI-ANGIOGENIC PROPERTIES ARE THERAPEUTICALLY ATTRACTIVE FOR MULTIPLE INDICATIONS

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Although mesenchymal stromal cells (MSCs) have been shown to be safe, their potency has recently been brought into question by failure of late-stage clinical trials to meet endpoints in multiple indications. Research groups and companies are therefore looking at different strategies to enhance MSCs to capitalize on the research investment. We have developed an innovative culture-based method that enhances MSC potency while maintaining MSC identity (US62/397572). Briefly, culturing engineering MSCs (ceMSCs) using a proprietary series of culture manipulations, post-expansion results in greater TSG-6 protein expression with (≥3 fold, $p < 0.05$, n=3) or without TNF-α stimulation (≥2 fold, $p < 0.05$, n=3) than unmanipulated MSCs (nMSCs); ceMSCs polarize monocytes/macrophages to M2 sub-types at a higher frequency than nMSCs (≥20 fold, $p < 0.05$, n=3). ceMSCs exhibit >19 fold increase in miRNA-16 (a known anti-angiogenic marker) levels vs. nMSCs, and inhibit proliferation of cancer cell lines (≥4 fold increases in G0/1 cell cycle phase and ≥3-fold decrease in G2/M cell cycle phase, $p < 0.05$, n=3) relative to nMSCs. Culture engineering can take place pre-freeze and is maintained during the cryopreserved state as evidenced by increased TSG-6 secretion (≥3 fold, $p < 0.05$, n=3) and decrease in CD4+ T helper (Th) cell proliferation, immediately post-thaw (≤1 fold, $p < 0.05$, n=3) by ceMSCs than nMSCs. The culture engineering can also take place immediately post-thaw, which restores anti-inflammatory properties of MSCs to levels observed with fresh, non-frozen ceMSCs (TSG-6 protein expression by post-thaw ceMSCs was ≥4 fold than nMSCs, $p < 0.05$, n=3). Culture engineering occurs after the MSC expansion stage, allowing scale-up of MSCs, coupled with enhancement of anti-inflammatory, anti-angiogenic properties, and is compatible with multiple, established manufacturing methods. We are developing a POC device for allogeneic MSC master cell banks to improve their anti-inflammatory and anti-angiogenic properties, post-thaw. This is important from a regulatory perspective as minimal alterations to existing manufacturing and control processes

are required. Altogether, we have developed a cost-effective, minimalistic process-based platform for activation of MSCs for various clinical indications.

T-2135

FOOTPRINT-FREE GENOME EDITING OF PATHOGENIC AND RISK VARIANTS IN HUMAN INDUCED PLURIPOTENT STEM CELL MODELS

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Genome engineering approaches in human induced pluripotent stem cells (iPSCs) has provided an opportunity to examine the contributions of pathogenic and risk alleles to molecular and cellular phenotypes. However, the practical application of genome editing approaches in human iPSCs has been challenging. We have developed an efficient genome editing platform that relies on allele specific gRNAs paired with a robust method for culturing and screening the modified iPSC clones that is highly robust. Using this approach, we have modified more than ten independent iPSC lines at seven pathogenic and risk loci. To determine whether this approach introduces off-target modifications, we compared the mutational burden across the edited iPSC lines using whole genome sequencing. The predicted off-target sites were unmodified in all edited iPSC lines. There was no significant difference in the numbers of variants that were detected in the edited and unedited iPSC lines. Additionally, no functional variants were observed across the edited iPSC lines. Thus, our CRISPR/Cas9 strategy does not specifically increase mutational burden. Furthermore, pathway analysis failed to identify an enrichment of genes within a given pathway across the edited lines. Thus, this bioinformatic analysis suggests that genetic variation between the iPSC lines represents de novo variants occurring randomly. Together, this platform will allow for efficient production of isogenic cell lines for disease modeling.

T-2137

AN ASCORBIC ACID DERIVATIVE ENHANCES THERAPEUTIC EFFECT OF MESENCHYMAL STEM CELLS ON AN ASTHMA ANIMAL MODEL

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Mesenchymal stem cells (MSCs) are multipotent stem cells with great potential for use in regenerative medicine. L-ascorbic acid (VitC) is generally used as a supplement of culture medium for MSCs to ensure their self-renewing and multipotency. However, VitC is extremely unstable in vitro since it is readily oxidizing to dehydroascorbate. Here, we show that ascorbic acid 2-glucose (AA2G), a stabilized VitC-derivative is beneficial to maintain the therapeutic potency of MSCs in vitro and in vivo conditions. Cultivation of human embryonic stem cells differentiated- (hES-MSCs) or human cord derived- (UC-MSCs) MSCs supplemented with AA2G increased the cellular proliferation and chemotactic activity toward PDGF-AA in concomitant with the activation of both MAPKp42/44 and AKT signaling cascades. Furthermore, the colony-forming unit-fibroblast and anti-inflammatory activities of both hES- and UC-MSCs were enhanced by AA2G supplement. In contrast to VitC, AA2G supplement has little toxicity even in high dosage. More importantly, a single transplantation of hES-MSCs cultured with AA2G into polyI:C-induced asthma animal exhibited the superior beneficial outcomes than naïve cells, based on i) decreased inflammation reaction around the bronchial and vascular area in lung tissues, ii) reduced infiltrated cellularity and inflammatory cells such as macrophages, neutrophils, and lymphocytes in bronchoalveolar lavage fluid (BALF), iii) increased IL-10 protein, an anti-inflammatory cytokine in BALF, and iv) increased the transplanted cells in lung tissue. Taken together, the present study shows that AA2G can stably increases the beneficial effects of MSCs for the treatment of asthma by enhancing their anti-inflammatory and engraftment capacity. By optimizing this protocol in future studies, MSC therapy might form a basis for clinical trials to treat human asthma and other intractable disorders.

T-2139

PRECONDITIONING WITH FAR-INFRARED IRRADIATION ENHANCES PROLIFERATION AND MIGRATION OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS VIA ERK/CXCR4 PATHWAYS

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For helping stem cell survive the inhospitable microenvironment of a damaged heart, we need to challenge at least two factors:1) stem cell(SC) boosters that modulate preconditioning of SCs; 2) strategies to enhance SC survival and engraftment of both endogenous and exogenous source of SCs. Far infrared therapy has been shown to exert positive effects in cardiovascular system. However, the biological effects of FIR on bone marrow-derived mesenchymal stem cells (BMSCs) are not understood. Herein, we investigated the effects of FIR generator with an energy flux of 0.13 mW/cm2 on BMSCs. The biological signatures underlying BMSCs by FIR exposure at 34°C for 50 min were measured using Ez-Cytox cell viability, EdU/Brd U assay, migration assay, western blot analysis, and immunofluorescence staining. Our results demonstrated that FIR induces BMSC preconditioning, which resulted in enhancement of cell survival against H2O2, proliferation, and migration. Furthermore, FIR exposure promotes the mRNA and protein expression of Nanog, Sox2, c-Kit, and Nkx2.5 with IGF-1 and CXCr4. Consistent with this, CXCr4 antagonist AMD3100 prevented the activation of CXCr4/ERK, as well as promotion of cell proliferation and migration by FIR. Together these findings provide the first evidence that FIR therapy can open combination strategies for improving the SC-based therapy for ischemic heart disease.

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T-2141

3D PRINTED HYDROGELS FOR SKELETAL MUSCLE DIFFERENTIATION OF HUMAN IPS CELLS

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Skeletal muscle atrophy caused by aging, disease, disuse and even microgravity exhibits a large impact on the health of the human body. However, with limited access to human skeletal muscle tissues, most physiological or pathological studies can only rely on animal models. Accumulating evidence also shows that the data from animal models may not completely represent the responses in the human body. Therefore, an in vitro culture system with human skeletal muscle may represent an alternative method to overcome the current problems we have, especially with creating a 3D culture system for this purpose. In our preliminary studies, we have developed protocols to induce skeletal muscle differentiation from human pluripotent stem cells. In this study, we tried to expand the culture system from 2D culture to 3D printed hydrogels for making 3D skeletal muscle tissues in vitro. Other in vitro studies have shown that the differentiation of mesenchymal stem cells may be affected by the stiffness of matrices. Thus, we plan to test the hypothesis that the differentiation of skeletal muscle from human iPS cells can be manipulated by changing the stiffness of hydrogel matrices. We have successfully modified and redesigned the open source 3D printer (Reprap Prusa I3) to be equipped with a syringe pump and heating element. This setup will allow us to load a thermal responsive hydrogel into the syringe in the liquid form by keeping the temperature between 50 and 60 C. After printing, the hydrogel can quickly solidify as it reaches room temperature or lower. In addition, we also designed an LED light source into the 3D printer model in order to photo-crosslink the hydrogel after printing when the hydrogel is mixed with photosensitizers. Therefore, we can manipulate the stiffness of the hydrogel by altering the gel concentration and the degree of photo-crosslinking. In conclusion, the unique design in the open source 3D printer will provide us a versatile platform to create novel 3D printed hydrogel structures for culturing human iPS cells and facilitating their differentiation.

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T-2143

IMPROVED HUMAN PLURIPOTENT STEM CELL EXPANSION IN STIRRED BIOREACTORS BY PROCESS CONTROL

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Human pluripotent stem cells are a unique source for the production of tissue-specific human cell types, fueling the development of advanced in vitro disease models and novel regenerative therapies. Most applications will require the constant supply of billions of cells generated by robust, economically viable bioprocesses. The

expansion of hPSCs as matrix-free cell-only aggregates in suspension culture (3D) is a potentially superior strategy for producing required cell numbers applying industry-compliant stirred tank bioreactor technology. We have recently established expansion and efficient cardiomyogenic differentiation of hPSCs in stirred tank bioreactors, demonstrating the universal utility of these systems for the mass production of hPSC-progenies. Moreover, daily medium replacement (termed repeated batch), which is typical for conventional tissue culture, was replaced by continuous medium exchange (termed perfusion). This advancement resulted in more homogeneous culture conditions, enabled significantly increased cell yields, and opened new possibility for process control. Here we have combined perfusion with the control of specific process parameters such as pH and the adaptation of medium components in the chemically-defined brew E8. The advanced process in 150 mL bioreactor scale resulted in superior conditions yielding >6 x 106 hPSCs / mL, which, to our best knowledge, is an unmet value in the field. Detailed analysis of other process indicators will also be presented, including comprehensive assessment of hPSCs' pluripotency status, differentiation potential, and the energy metabolism. Together, the study highlights the enormous potential for process development in hPS cell manufacturing, particular by using well monitored and controlled bioreactor systems, which also facilitates straightforward upscaling.

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T-2145

STANDARDIZING AND DE-RISKING THAWING OF CRYOPRESERVED HUMAN PBMCS

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Stem cell therapies offer a potentially historic advance in how we practice medicine, yet present unique translational challenges in the areas of patient safety and efficacy, due to their inherently variable nature. For logistical reasons, most cell therapy products are cryopreserved, then thawed at point-of-care. Thawing is considered one of the most critical steps for live therapeutics post-manufacturing, since non-standardized thawing methods can lead to loss of viability and function, lowered proliferation, altered cell subset recovery, and increased cellular stress. Furthermore, "traditional" water bath thawing methods expose therapeutic cells to contamination risk, variable thaw times, and subjective thawing endpoints. Human peripheral blood mononuclear cells (PBMCs) encompass a wide variety of multipotent progenitor cell populations, and are consequently prized for their ability to differentiate into many different cell types. Their therapeutic value is currently being evaluated in over 900 clinical trials, for applications ranging from autoimmune disease, to tissue regeneration and repair. In this study, we address the need to de-risk and standardize thawing by comparing thawing of cryopreserved PBMCs with the ThawSTAR® water-free automated thawing platform to conventional water bath thawing. Cell recovery, viability, and functionality were analyzed via trypan blue exclusion and IFN-γ ELISpot assay. Post-thaw average viability, post-thaw average recovery, and post-rest average viability and function as measure by IFN-γ ELISpot are comparable for cells thawed using the water bath or ThawSTAR® platform. Interestingly, post-rest (16-24 hours) recovery is greater for PBMCs thawed using the ThawSTAR® automated thawing platform than for PBMCs thawed in a water bath. Therefore, the ThawSTAR® platform exposes cells to less stress during the thawing procedure than water bath thawing, in addition to eliminating water borne contamination risk. We conclude that thawing with the ThawSTAR® automated cell thawing platform increases longer-term cell health and reduces risk, and is therefore more suitable to thawing cell therapy drug products than the water bath.

T-2147

DEVELOPMENT OF A GLYCOSYLATION-INDEPENDENT, IHC-VALIDATED RECOMBINANT MONOCLONAL ANTIBODY AGAINST THE CANCER STEM CELL MARKER CD133

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The cancer stem cell hypothesis posits that a population of self-renewing tumor-initiating cells, termed Cancer Stem Cells (CSCs), may be responsible for driving tumor heterogeneity, metastasis, therapeutic resistance and/or tumor relapse. Tools to identify and characterize putative CSCs are therefore of significant value for the cancer research community. CD133 is a 5-transmembrane (5-TM) cell surface glycoprotein that shows elevated expression in putative CSCs from multiple tumor types. Numerous studies have used antibodies directed against

CD133 to isolate putative CSCs for characterization, in vitro culture, transplantation and drug discovery studies. However, the most commonly used antibodies used to study CD133+ cells are raised against glycosylated CD133 epitopes; this is problematic because the glycosylation status of CD133 varies in response to environmental conditions (e.g., hypoxia) or cell differentiation status. Furthermore, available anti-CD133 antibodies have not been rigorously validated for immunohistochemistry, which is critical for understanding CSC biology in situ. To address this problem, we have developed a recombinant rabbit monoclonal antibody that targets an extracellular, glycosylation-independent epitope of CD133. This reagent has been rigorously validated in Western immunoblot and immunohistochemistry, where it demonstrates robust and specific staining of CD133 protein across diverse cell and tissue types. This reagent will enable accelerated progress in elucidating the role of putative cancer stem cells in the development, metastasis, therapeutic resistance and relapse of tumors.

T-2149

ENHANCED MIGRATORY ACTIVITY AND IN VIVO ENGRAFTMENT RATE OF HUMAN MESENCHYMAL STEM CELLS AFTER MICROENCAPSULATION IN AND TRANSMIGRATION THROUGH A COLLAGEN BARRIER

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Human mesenchymal stem cell (hMSC)-based therapies has extremely low engraftment rate. Strategies enhancing the migratory activities of these cells are critical to improving the engraftment rate and hence translational potential of MSC-based therapies. hMSCs are heterogeneous mixtures of multiple cell types, which are different in morphology, phenotype and functional properties including migratory activities. Here, we ask a question that whether hMSCs transmigrating through a collagen barrier have better migratory activities and in vivo engraftment rate. In brief, hMSCs were microencapsulated into reconstituted nano-fibrous collagen meshwork and allowed to transmigrate through this collagen barrier. Detailed investigations on the transmigrated MSCs including phenotype, proliferation, differentiation, migratory activity, integrin adhesion, MMP secretion and in vivo engraftment rate were conducted, in comparison with a number of control groups. Results showed that using a transwell assay, these transmigrated significantly enhanced migratory activities as compared to the controls, both in serum free medium and in medium containing major chemokines SDF-1 and Fractalkine for hMSCs. Among other mechanisms, the transmigrated hMSCs showed a dramatic increase in their contractility as measured by the traction force measured by a protein micropillar array-based traction force assay. Using a partial hepatectomy model in NOD/SCID mice, after tail vein injections of hMSCs, the transmigrated hMSC group showed a significantly higher engraftment rate comparing with the control group. Further investigations to delineate the detailed mechanism of contractility change is warranted.

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T-2151

IL-6 IS ABLE TO COMPENSATE THE REDUCED MIGRATORY ACTIVITY OF MSC AFTER SM EXPOSURE

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Mesenchymal stem cells (MSC) are important for the regeneration of wounded skin. Essential requirement for a MSC driven wound regeneration is an active migration into the wounded area. Is the skin wounded for example by the bi-alkylating agent sulfur mustard (SM), patients show chronic wounds with an impaired wound healing for weeks till month. As for patients with chronic wound healing disorders is known that migratory capacity of MSC is altered we therefore wanted to investigate how SM affects MSC and if these findings might constitute a better understanding of the effect of sulfur mustard gas with respect to skin wounds. Initially we had observed the strongest impact from SM to the secretion of IL-6, which was significant, reduced. Beside that we also observed a significant reduction of the migratory activity of MSC under influence of this alkylating agent in a dose dependent manner using a modified Boyden Chamber system. To investigate a further aspect, we analyzed the effect of SM exposure on the secretome of MSC. Using slide-chip arrays we investigated the semi-quantitative secretion of 275 cytokines. Out of these we identified the strong impact from SM to the secretion of IL-6, which was significant reduced. As IL-6 is known to enhance the migratory activity of MSC we suggest a direct link between the inhibitory effect of SM on MSC migration and the secretion of IL-6. To confirm this hypothesis, we repeated the SM exposure experiments while adding additional IL-6 in Boyden Chamber experiments. In deed IL-6 could repeal the inhibitory effect of SM onto MSC migration. These findings demonstrate a direct dependence from the anti-migratory effect of SM to MSC via IL-6. These results can indicate potential therapeutic

opportunities for using IL-6 to reduce chronic wound healing after exposure of SM.

T-2153

IMPROVED TALEN FOR KNOCK-IN APPLICATIONS WHERE CRISPR FAILS

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Gene editing makes possible the targeted modification of nucleotide sequences within a genome, and is achieved by using guided endonucleases to create a double-stranded break in DNA; inducing a mutation. Transcription Activator-Like Effector Nucleases (TALENs) are an existing gene editing technology that can disrupt a gene (knockout) or insert a new sequence (knock-in via addition of homologous donor DNA). Historically, CRISPR-Cas9 has demonstrated better knockout efficiency than TALENs and is less expensive. The use of Cas9 is constrained by the requirement of a Protospacer Adjacent Motif (PAM) sequence (NGG) to bind. In addition, homologous recombination repair efficiency is a function of nuclease cleavage efficiency as well as proximity of the cleavage sites to the desired edit position (< 10 bases). Sometimes the available Cas9 cleavage site is too distant from the desired edit site, resulting in poor repair efficiency. TALENs do not require a PAM site to bind and the advent of T-less TALENs removes the 5' T requirement. Since TALENs can be engineered to cut at any target site in a genome, they can be used to cleave the DNA at the knock-in site. Recent modifications to the assembly process have allowed for TALEN production that is faster and less expensive, making TALENs cost competitive with CRISPR-Cas9. This study tested 13 human genes, including five genes associated with pathogenic single nucleotide polymorphisms (SNPs) without a nearby PAM site. Each gene was tested using five TALEN pairs targeted to the exact modification site, as well as two and four nucleotides upstream and downstream. Three immortalized cell lines and iPSCs were tested to determine the cleavage efficiency of TALENs and the number of TALEN pairs needed to be screened to obtain the desired cleavage at any given target site. Then a SNP repair donor was supplied with the most efficient TALENs to measure the repair efficiency compared to the proximal CRISPR sites. In addition, in an effort to improve overall cleavage efficiencies, modifications to the TALEN architecture were evaluated in traditional cell lines and iPSCs. This approach provides TALENs as a complementary tool for efficient knock-in rescue of pathogenic SNPs and N- or C-terminal tagging of epitopes or fluorescent proteins via HDR.

T-2155

CORD BLOOD CELL-DERIVED IPSCS AS A NEW CANDIDATE FOR CHONDROGENIC DIFFERENTIATION AND CARTILAGE REGENERATION

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The native articular cartilage lacks the ability to heal. Currently, ex vivo expanded chondrocytes or bone marrow-derived mesenchymal stem cells are used to regenerate the damaged cartilage. With unlimited self-renewal ability and multipotency, human induced pluripotent stem cells (hiPSCs) have been highlighted as a new replacement cell source for cartilage repair. Still, further research is needed on cartilage regeneration using cord blood mononuclear cell-derived hiPSCs (CBMChiPSCs). Human iPSCs were generated from CBMCs using sendai virus. The characterization of CBMC-hiPSCs was performed by various assays. Embryonic bodies (EBs) were obtained using CBMC-hiPSCs, and outgrowth cells were induced by plating the EBs onto a gelatin-coated plate. Expanded outgrowth cells were detached and dissociated for chondrogenic differentiation. Outgrowth cells were differentiated into chondrogenic lineage with pellet culture. Chondrogenic pellets were maintained for 30 days. The quality of chondrogenic pellets was evaluated using various staining and genetic analysis of cartilage specific markers. Reprogramming was successfully done using CBMCs. CBMC-hiPSCs (n=3) showed high pluripotency and normal karyotype. Chondrogenic pellets were generated from the outgrowth cells derived from CBMC-hiPSC EBs. The generated chondrogenic pellets showed high expression of chondrogenic genetic markers such as ACAN, COMP, COL2A1, and SOX9. The production of extracellular matrix (ECM) proteins was confirmed by safranin O, alcian blue and toluidine blue staining. Expression of collagen type II and aggrecan was detected in the accumulated ECM by immunohistological staining. Chondrogenic pellets showed low expression of fibrotic and hypertrophic cartilage marker, collagen type I and X. This study reveals the potential of CBMC-hiPSCs as a promising candidate for cartilage regeneration.

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T-2157

AN ENGINEERED ANALYSIS ON RELATIONSHIP BETWEEN STRESS FROM MEDIUM FLOW AND HIPSC AGGREGATES BEHAVIORS IN VARIOUS SHAKING SUSPENSION CULTURE

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Suspension culture is one of the most promising culture methods for cell mass production. In the case of human induced pluripotent stem cells (hiPSCs), they are known to form aggregates during suspension culture. Although there are many reports about suspension-based mass production of PSCs, there are few reports on the relationship between stress from medium flow and hPSC behaviors such as aggregation, growth, movement and pluripotency. In this research, we developed the system with a high-speed camera for capturing and tracking movements of particles in shaking vessels. By the capturing system, we captured and tracked the stained beads in a 6-well plate with various shaking conditions: 60, 90 and 120 rpm of rotation and reciprocation. From tracking data, we calculated their accelerations in order to figure out their stress from medium flow. According to the calculation of the acceleration, their stress increased with higher rpm in both shaking methods. The calculation also revealed that their stress in rotary shaking was smaller than that in reciprocating even in same rpm. Remarkably, the periodic acceleration shift was observed only in reciprocating condition. According to actual hiPSC suspension culture, at 60 rpm of both shaking methods, they formed a huge aggregate approximately larger than 1 mm, which caused low growth. At 90 rpm, cell growth reached highest in both shaking methods, and the growth decreased at 120 rpm probably because of high stress from medium. In addition, at the same rpm, their growth in rotary shaking culture was higher than that in reciprocating culture. This growth decrease could be eased by supplementing bovine serum albumin (BSA). Pluripotency analysis by flow cytometry showed that there was no significant difference of pluripotency marker expression among various shaking conditions. These facts indicate that stress from medium damage hiPSCs in suspension culture, which can be relieved by supplementing proteins such as BSA and did not affect their pluripotency. This knowledge can be useful for designing suspension-based culture system with lower stress. Now we are investigating on and will present the detailed relationship between medium flow and forming aggregates.

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T-2159

IMPROVED CELL CULTURE STRATEGIES FOR EFFICIENT GENOME EDITING IN HUMAN PLURIPOTENT STEM CELLS

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CRISPR/Cas9-mediated genome editing allows efficient genetic manipulation of mammalian cells. However, major bottle-necks in applying this exciting technology to human pluripotent stem cells (hPSCs) are optimizing the delivery of the CRISPR/Cas9 tools, improving the cell culture workflow for efficient screening of correctly genome-edited clones, and ensuring that the stem cells retain their pluripotency after the screening process. We addressed some of these issues by comparing cell culture methodologies: non-enzymatic cell-cluster passaging versus enzymatic single-cell passaging, different types of media and substrates to support feeder-independent cell culture, and different transfection reagents. To facilitate the enrichment of transfected hPSCs through a cell-surface tag without resorting to drug selection, we generated an all-in-one vector that expresses EF1a::CD4-P2A-Cas9 and U6::sgRNA for genome editing. We show that cells adapted to single-cell passaging are more amenable to nucleofection with the all-in-one vector, and that they can be magnetically sorted using CD4-binding beads. We assessed both knock-out and knock-in efficiency by targeting a constitutively expressed GFP transgene in an hPSC line. In sum, we present an improved workflow for efficient genome editing in hPSCs without the need for fluorescence- or drug resistance-based selection.

T-2161

CHRONIC SENESCENT MESENCHYMAL STEM CELLS COULD BE THE CAUSE OF CHRONIC WOUND HEALING DISORDERS AFTER SULFUR MUSTARD EXPOSURE

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Chronic wound healing disorders after sulfur mustard (SM) exposure are not completely understood. Bone marrow derived mesenchymal stem cells (MSCs) are essential for wound healing. We hypothesize that MSC disturbances are involved in the process of

disrupted wound healing after SM exposure. MSCs in chronic senescence may provide the "cellular memory" responsible for such wounds. If MSCs undergo senescence after SM exposure they might be able to imprint senescence onto other MSCs. Such SM-induced chronic senescent cells would be able to escape the immune system, persist over long time periods and display a senescence-associated secretory phenotype (SASP). Human mesenchymal stem cells were isolated from bone marrow of femoral heads, which were obtained during total endoprosthesis of the hip joint. Isolation efficiency was confirmed by FACS measurement and senescence was determined by X-gal staining of senescence-associated β-galactosidase (SA-β-gal). Expression levels were determined by Western blot. Senescent MSCs are clearly visible 6 days after acute SM treatment for the first time and the number of senescent cells as well as the intensity of the staining increases until day 12. At this time, it seems that all cells are senescent at the four tested SM concentrations (1, 10, 20 and 40 µM). Prolonged incubation of these cells results in no obvious change in senescent state up to day 21 for the cells treated with high SM concentrations. In contrast, 1 µM SM treatment seems to be less effective because there are many non-senescent cells visible. Morphological changes like flattening and increased expression of senescence markers such as p16INK4a were observed. In conclusion, we could demonstrate that the acute treatment of MSCs with SM resulted in chronic senescence after 12 days and was stable thereafter. Senescence markers such as SA-β-gal and p16INK4a expression were validated. This research is providing new insight into the pathomechanism of chronic wounds after SM exposure which should allow the development of alternative therapeutic strategies on the long term.

T-2163

SUPPORTING EXPANSION OF HUMAN MESENCHYMAL STEM CELLS (HMSC) USING THERMO SCIENTIFIC NUNC CELL FACTORY SYSTEMS

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Human Mesenchymal Stem Cells (hMSCs) based therapies are at different stages of clinical investigation for a wide range of applications, utilising e.g. either the immunomodulatory properties of hMSCs in "Graft versus Host Disease" and Crohn's disease or differentiated hMSCs for tissue repair. To meet the demand of cell number, there is a need to develop tools for hMSCs expansion along with the maintenance of multipotency. The poster will illustrate how Thermo Fisher Scientific can support customers in the process of expanding hMSC in either small cultureware– or in larger-scale Thermo Scientific™ Nunc™ Cell Factory™ systems (CF) using classical or chemically defined medium. Overall, consistent performance was observed between T75 flasks and CF1 trays for either classic, serum-containing medium (SCM) or MesenPRO™ reduced-serum medium (SRM) and cultured the cells over four passages. The cell yield obtained per cm2 also highlights the comparable performance between the laboratory- and the large-scale culture vessels. For each media type, the yield/cm2 differed only by 10% between T75 flasks and CF1 trays with maintenance of overall cell viability greater than 96%. By increasing the surface area contained within the CF system from a single-tray to a 10-tray vessel, true scalability of hMSC cultured in SCM was observed. hMSC were seeded at a density of 2,000 cells/cm2, 8 million cells (8.1 E06) were harvested from a single tray of a CF system following 4-day culture. By the time cells had been scaled up to a 10-tray CF system using the same culture paradigm, 80 million cells (8.27E07) were harvested. This 10-fold increase in the yield corresponds to the 10-fold increase in available surface area between a single-tray and a 10-tray CF system. The scalability of expanding hMSC in SCM within CF system is reflected by the consistent growth rate of hMSC with maintenance of multipotency, when scaled up from a single-tray to a 10-tray CF system.

T-2165

EXPANSION OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN STIRRED SUSPENSION CULTURE

Lawson, Tristan[1], Kwok, Chee Keong[2], Ueda, Yuichiro[3], Verma, Anjali[1], Pease, Megan[1], Kadari, Asifiqbal[3], Guenther, Katharina[3], Schnitzler, Aletta[1], Heron, Antoine[4], Rook, Martha[1], Edenhofer, Frank[3] and Murrell, Julie[1]
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The therapeutic potential of human induced pluripotent stem cells (hiPSCs) is explored in a large array of indications, ranging from acute myocardial infarction to diabetes. The inefficiencies in some current differentiation protocols combined with the large numbers of cells recommended for clinical scale tissue engineering warrant the use of systems that are capable of generating large batches of hiPSCs in a controlled manner. Prior studies have shown the ability to culture hiPSCs in stirred suspension culture, however the culture vessels have mostly been limited to small scale spinner flasks with no inherent feedback control of pH and dissolved oxygen (DO). In this study, hiPSCs were seeded

as single cells in 125mL spinner flasks, and agitated at a rate that would support aggregate formation. This culture method circumvented the need for a substrate or microcarriers. After growing the aggregates for up to 7 days, they were dissociated into a single cell suspension and cultured for an additional 7 days in a single-use 3L bioreactor. Overall a 125 fold expansion was attained after a combined 14 day culture. After aggregate formation and growth in the bioreactor, the hiPSCs were replated onto planar culture for characterization assays which included immunocytochemistry of iPSC colonies, flow cytometry, and differentiation toward tissues of the embryonic germ layers. The hiPSCs retained expression of the pluripotency markers and formed tissues of each of the three germ layers. The results demonstrate the potential of hiPSC production in controlled stirred suspension systems that can support the production of large batches of cells for research and clinical applications.

T-2167

LONG-TERM MAINTENANCE AND MASSIVE PRODUCTION OF HUMAN PLURIPOTENT STEM CELL WITHOUT GENOMIC INSTABILITY USING ROS SYSTEM

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Human Pluripotent Stem Cells (hPSCs) can be propagated and expanded in vitro indefinitely, and provide a potentially inexhaustible source of stem cells for human therapy. Various cell types derived from hPSCs would provide robust cellular platforms for disease modeling, drug screening, and toxicological testing. The key issue for their future clinical and pharmacological applications is the generation of large numbers of clinical-grade hPSCs through in vitro cell culture. Many efforts to produce large numbers of hPSCs for cell therapy and drug development have developed but the current culture methods are not satisfying all requirements for simple, robust, cost-efficient and safe production of hPS cells. To overcome these above fundamental limitations, we deploy reactive oxygen species (ROS) generated by plasma jet as an alternate source of novel hPSC culture. Over the last few years, studies have suggested that oxidative stress plays a role in the regulation of hematopoietic cell homeostasis. An optimal plasma jet treatment generates a minor amount of ROS in cells and culture medium. The generated ROS mitigates markedly dissociation-induced apoptosis of hPSC and increases substantially attachment and proliferation of hPSCs. The continuous optimal ROS stimulus on hPSCs facilitates fast colony formation in vitro culture. In addition, ROS stimulus to hPSCs results most likely in the increase of beginning ratio of cell attachment, cell viability, and pluripotency gene expression such as Oct4, Nanog, and tra1-60. They can be differentiated into various cell types in vitro and in vivo. hESC are exposed to higher lactate concentrations, meanwhile, leading to a lower medium pH when grown in higher densities. The acidification by accumulation of higher lactate concentrations in culture medium impacts on their genomic stability and increase DNA damage in hPSCs. Interestingly, the optimal ROS generation protects a drop of pH in culture medium and mediates safe genome stability in long-term culture of hPSCs. Here, we demonstrate a direct correlation between ROS production, long-term maintenance of hPSC, and the occurrence of genomic instability. Our study will fulfill all requirements such as simple, robust, cost-efficient and safe production of hPS cells by taking advantage of ROS in automated GMP facility.

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T-2169

MULTIWELL MICROELECTRODE ARRAY TECHNOLOGY FOR THE EVALUATION OF HUMAN IPSC-DERIVED CARDIOMYOCYTES

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The flexibility and accessibility of induced pluripotent stem cell technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Indeed, rapid advances in stem cell technology have led to widespread adoption for the development of in vitro models of cardiomyocyte electrophysiology to be used in screening applications in drug discovery and safety. Specifically, international drug safety initiatives, such as CiPA and JiCSA, underscore the interest and utility of human iPSC-derived cardiomyocytes. An important element of these initiatives, and others, is the continued improvement and optimization of the commercial cardiomyocyte product, including the differentiation protocol, the manufacturing procedures, and the consumables (e.g., media) required for development. Here, we present data supporting the use of the Maestro multiwell microelectrode array (MEA) technology as an

efficient approach to quantification and optimization of human iPSC-derived cardiomyocyte production. A planar grid of microelectrodes interfaces with cultured cardiomyocyte networks embedded in the substrate of each well, such that the electrodes detect the raw electrical activity propagating across the syncytium. Quantification of the cellular electrophysiological activity, including spike amplitude and field potential duration, provided information on the depolarization and repolarization of the cardiomyocytes action potential. The analysis was performed across development conditions, wells, and plates to evaluate the reliability of the cardiomyocyte MEA assay. These results support the continued development and use of human iPSC-derived cardiomyocyte assays on the Maestro multiwell MEA technology for high throughput drug discovery and safety assessment.

T-2171

PRE-SELECTION OF MOUSE MESENCHYMAL STEM CELLS BY MOLECULAR IMAGING IN THE TREATMENT OF INFLAMMATORY DISEASE

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Bone marrow stromal stem cells (BMSCs) are potent modulators of immune responses. However, they are heterogeneous in their phenotype and functions, and may have multiple origins. We aimed to pre-select BMSCs and try their effects in inflammatory disease treatment. For our studies, nestin-GFP+ and C57Bl6 mice were used. After flushing the bone marrow, CD45+/ Ter119+/ CD31+ cells were removed by magnetic separation and the remaining cells were analyzed for negative, as well as CD140a and sca-1 positive markers using flow cytometry and sorting. Separated BMSCs were plated in vitro with macrophages and stimulated by zymosan (1ug/mL) for 6-18h. Pro-inflammatory TNFa and anti-inflammatory IL-10 levels were measured from the supernatants by ELISA. BMSCs were also injected ip. into a zymosan-induced peritonitis model. After 18h,peritoneal lavages were performed for cell counting and flow cytometry analysis. Fluorescence molecular imaging positively separated the osteo/ adipogenic plastic adherent CD140a+ and sca-1+ stromal stem cell population from other bone marrow cells. BMSCs reduced macrophage TNFa production ($p < 0.01$) and up-regulated their IL-10 production ($p < 0.01$) in a cell-number dependent manner. In the in-vivo peritonitis assay, separated stromal cells also reduced the infiltrating inflammatory polymorphonuclear leukocyte numbers ($p < 0.05$). Fluorescence molecular imaging is a suitable tool for bone marrow stromal cell characterization. Separated BMSCs proved to be anti-inflammatory both in co-cultures by modulating macrophage cytokines and in a peritonitis assay by decreasing the inflammatory cell numbers.

T-2173

ESTABLISHMENT OF NOVEL HUMAN PLURIPOTENT STEM CELLS CULTIVATION METHODS

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Handling of human pluripotent stem cells (hPSCs) is hard work because it is required with well-experienced cultivation technique and changing medium every day. Previously we reported that suppressed proliferation and maintained an undifferentiated PSCs modified culture media containing D-xylose (11th ISSCR). Therefore we investigated method that novel cultivation for avoidable cell handling of nonworking day by using the modified medium (Xylose medium). In first, hiPS cells (hiPSCs) were cultured on inactivated mouse embryonic fibroblasts (MEFs) with hiPS growth medium, and then culture media switched to Xylose medium. Analysis of real-time PCR and immunofluorescence showed that after cultivation in Xylose medium, the cells were maintained high expression levels of undifferentiated markers. Subsequently, the hiPSCs were engrafted into renal capsule of immuno-compromised mice in renal capsule to generate teratomas. Within the resulting tumors derived from the hiPSCs, the various derivatives of the three primary germ layers. The hiPSCs cultured with Xylose media had potency of embryoid body formation, and high efficiency of differentiation into definitive endoderm. It is anticipated that this medium contribute to reduce the burden of cell handling of researchers in stem cell research and regenerative medicine, because it is applicable to conventional on-feeder culture of hiPSCs.

POSTER SESSION II-EVEN 19:00 – 20:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

T-1002

CONDITIONED MEDIUM OBTAINED FROM AMNION-DERIVED MESENCHYMAL STEM CELLS PREVENTS THE ACTIVATION OF KELOID FIBROBLASTS

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Mesenchymal stem cells (MSCs) are a valuable cell source in regenerative medicine, and conditioned medium (CM) obtained from MSCs (MSC-CM) reportedly inhibits inflammation. Keloids are characterized by abnormal proliferation of fibroblasts in response to inflammation. In this study, we evaluated the effect of amnion-derived MSC-CM on the activation of keloid fibroblasts. Keloid (N=7), mature (N=3) and normal (N=3) fibroblasts were harvested from patients. Cells were cultured and stimulated with TGF-β, and the effect of human amnion-derived MSC-CM on the activation of fibroblasts was investigated by MTS assay, quantitative real-time PCR and immunofluorescence staining. We also analyzed the effect of 5× concentrated MSC-CM on the activation of fibroblasts. MSC-CM significantly suppressed the TGF-β-induced upregulation of αSMA, SM22a, and collagen-I in keloid fibroblasts, but not mature and normal fibroblasts. The expressions of collagen-III, MMP2 and cell proliferation were not affected by MSC-CM in all fibroblasts. Concentrated MSC-CM had tendency to attenuate the activation of keloid fibroblasts as compared to standard MSC-CM. In conclusion, MSC-CM prevented activation of keloid fibroblasts. Amnion-derived MSC-CM would be a promising treatment for refractory keloid.

T-1004

COST EFFECTIVE CRYOPRESERVATION OF WHOLE HUMAN UMBILICAL CORD TISSUE

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Umbilical cord stroma has been widely accepted as an easily available, non-ethical concerned, and promising source of multipotent stem cells for regenerative medicine. The low cost effective cryogenic storage of umbilical cord tissue for banking will have a broad commercialising potential for autologous transplantation or scientific purposes. A cocktail cryopreservation solution (CS) contains penetrating cryopreservation agents (CPAs), sugar and macromolecular compounds has been developed. The whole umbilical cord tissue was frozen followed a programmed CS loading and cooling procedure, that stored in -80°C freezer overnight and then transferred to liquid nitrogen and stored for up to two weeks. The cryopreserved cord tissues were thawed by a three-step protocol to remove the CPAs. The earliest outgrowth of mesenchymal stromal cells was observed from day 5 after plating and incubating in 37°C incubator. These cells displayed the classic spindle shape and similar proliferation rate as the cells derived from fresh tissue. Flow cytometry analysis showed that these cells expressed typical MSC surface markers, including: CD73, CD90 and CD105 but negative for endothelial marker CD31. This study proved that reserving the viability of cells in cryopreserve whole umbilical cord tissue could be effectively achieved by using a proper penetrating CPA in well osmotically balanced solution and following a programmed cooling procedure.

ADIPOSE, MUSCULOSKELETAL, AND CONNECTIVE TISSUE

T-1006

PROLIFERATION AND OSTEOGENIC DIFFERENTIATION POTENTIALS OF HUMAN BONE-DERIVED CONNECTIVE TISSUE PROGENITORS UNDER HIGH GLUCOSE CULTURE CONDITION

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Regeneration of connective tissues depends on connective tissue stem/progenitor cells (CTPs) that are resident in native tissues . The concentration and prevalence of CTPs vary between individuals and may change as a result of aging or disease. The response of these cells with respect to proliferation, migration, differentiation and survival depends on the number, intrinsic biological potential of the CTPs and their responses to local microenvironment. Diabetes has been associated with delayed fracture repair, accelerated age-related bone loss, increased risk of osteoporosis, and accumulation of adipocytes in the marrow cavity, at the expense of bone and hematopoietic tissues. Glucose concentration may affect cell proliferation and the relative tendency of CTPs to choose an osteogenic vs. adipocytic fate. The purpose of this study was to: 1) characterize the effect of high glucose (HG – 25 mM) on colony forming efficiency (CFE), proliferation, and osteogenic differentiation of primary human bone-derived CTPs; 2) assess whether the HG induces an autophagy; and 3) characterize whether heparin can modulate the effects of HG. Cells were isolated from the trabecular surface of cancellous bone tissue from the proximal femur of 7 non-diabetic patients undergoing hip arthroplasty in an IRB approved protocol. We assessed: 1) colony formation and expression of an osteogenic marker using ColonyzeTM software to determine CTP prevalence (PCTP), colony metrics, cell counts, and alkaline phosphatase (AP) area fraction; and 2) gene expression of osteogenic (Runx-2, AP, osteocalcin), adipogenic (C/EBP-α and FABP-4) differentiation markers and autophagy (Cyclin D3, Beclin1) markers. Colony formation, proliferation and AP expression were decreased in HG conditions, and markers of autophagy were higher. Contrary to predictions heparin also decreased colony formation and proliferation. HG conditions did not affect other colony metrics or osteogenic/adipogenic differentiation markers. This study demonstrates significant effects of HG conditions on human bone-derived CTPs. Large variations were observed between subjects with respect to response to HG which may have important implications in understanding the interaction between glucose control in diabetes and the preservation of bone and bone marrow health.

Funding Source: Department of Defense - W81XWH-12-2-0118

T-1008

IDENTIFICATION OF A LATENT RESIDENT PROGENITOR POPULATION IN THE ADULT TENDON

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Tendons connect and transfer force between the muscles and bones of the body, making them highly prone to injury. These injuries encompass acute tears and rupture to chronic degradation and deterioration of the tissue. Tendons are largely composed from type I collagen and cells that are believed to respond to growth and tendon injuries. However, we have limited knowledge about the identity and molecular mechanisms that regulate these cells. In our study, we quantified the adult tendon cell turnover rates from postnatal growth stages to adulthood, and we identified a unique cell population that proliferated and responded to tendon injury. In order to determine the cell turnover rates during growth, homeostasis and injury we used the doxycycline (Dox) inducible TetO-H2B-GFP (Col1a1:tetO-H2B-GFP; ROSA:rtTA) reporter mice that have previously been used to quantify the proliferative history of a tissue, and BrdU labeling. Together, we found relatively higher proliferation rates during early postnatal stages prior to 1 month of age, and significantly lower turnover rates (between 1-5%) after 1 month. Interestingly, we found a distinct subpopulation of Axin2-labeled cells in the adult tendon during homeostasis. To assess proliferation during healing, we injured the Achilles tendon and continuously pulsed the mice with BrdU for one month after the injury. We found more than 30% of the cells labeled with BrdU at the site of injury, indicating a tendon cell population had divided during the healing response.

Lineage tracing of the Axin2+ cells showed that they proliferated following injury and were the predominant cell population at the healed site, suggesting a special role for these cells in tendon healing. Moreover, deletion of Porcupine, a factor necessary for Wnt secretion, in the Axin2+ cells resulted in a severely impaired healing response. Therefore, we believe that the Axin2+ cells serve as a unique progenitor cell pool that responds to tendon injury and their activity is regulated by the Wnt pathway. Our future experiments are focused on further exploring the function of Axin2+ progenitor cells and Wnt signaling in tendon injury repair.

Funding Source: Human Frontiers Science Program (HFSP)

T-1010

A ZEBRAFISH HIGH-THROUGHPUT CHEMICAL SCREEN IDENTIFIES TENDON PROMOTING DRUGS

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Tendons are an essential component of musculoskeletal system and transmit the force between the muscles and bones. Tendon injuries affect a significant portion of population and have a slow and limited repair process. Therefore, improving the healing response after injury represents a promising therapeutic strategy. However, currently, there are limited treatments for tendon injuries, which stems from a poor understanding of tendon biology. To discover novel regulators of tendon development, we took advantage of the zebrafish system and performed a large-scale chemical screen. Since tendon lineage promoting factors and developmental regulators have been shown to be effective at improving injury outcomes in animal models, our zebrafish screen has the potential to identify regulators of tendon formation that could be used in regenerative strategies for tendon repair. Using a zebrafish double transgenic line that labels tendon (mcherry) and cartilage (GFP) and a blastomere cell culture system, we screened 7569 small molecules from 11 bioactive drug libraries. We identified 111 hits that altered mcherry and GFP signal. Among these 111 hits, we selected and validated 16 with the highest tendon promoting abilities. qPCR analysis of the hit compounds in zebrafish blastomere cells and whole embryos revealed increased expression of tendon genes, including the earliest progenitor marker scxa (scleraxis a) and differentiation marker tnmd (tenomodulin). To demonstrate a conserved role for these compounds in human cells, we treated adult human mesenchymal cells (MSCs) with the hit compounds. For 12 of the 14 hits, we observed increased expression of tendon and/or cartilage markers, suggesting these drugs have conserved tendon and cartilage promoting activities in human cells. Our next experiments are focused on testing the hits on human pluripotent stem cells to determine if they can promote tendon differentiation. Taken together, the compounds that we discover will lead to the identification of novel pathways involved in tendon development. Our goal is to identify tendon promoting pathways that can be harnessed for the development of regenerative treatments to improve tendon healing.

T-1012

MITOCHONDRIAL DYSFUCTION MEDIATES THE IMPAIRED IMMUNOMODULATORY ABILITY OF ADULT HUMAN MESENCHYMAL STROMAL CELLS

Kizilay Mancini, Ozge[1], Lora, Maximilien[2], Servant, Marc[3] and Colmegna, Ines[2]
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Mesenchymal stromal cells (MSC) are multipotent cells capable of homing to injured tissues and possess powerful immunomodulatory capacities. The latter led to their use in clinical trials for a variety of autoimmune and inflammatory diseases. The results of such trials had been conflicting. Among the factors potentially contributing to those inconsistencies is the inter-study variability in MSC donors' age. Indeed, MSCs from older individuals display decreased therapeutic efficacy and have altered immunomodulatory function in vitro (i.e. MSC-mediated inhibition of CD4+ T cell proliferation in allogeneic co-cultures). The mechanisms underlying the impaired immunomodulatory function of aged MSC remain elusive. The aim of this study was to determine the contribution of altered mitochondrial function to the age-associated reduction of MSC's immunomodulatory activity. Adipose tissue-derived MSCs were isolated from adults (aMSCs; ages 38-79) and pediatric (pMSCs; ages 7-21) donors. aMSCs produced less ATP (n=5, p=0.03) and had higher levels of intracellular- (n=15, p=0.002) and mitochondrial- (n=10, p=0.04) reactive oxygen species (ROS). Mitochondrial ROS levels inversely correlated with MSC immunopotency (n=24, R square=0.31, p=0.004). The expression of the antioxidant enzymes catalase (n=5, p=0.4) and superoxide dismutase (n=5, p=0.3), were similar in aMSC and pMSC. Importantly, oligomycin-induction of mitochondrial dysfunction in pMSCs resulted in an aMSC-like phenotype with increased levels of

pro-inflammatory cytokine (i.e., IL-6, IL-8 and MCP-1) secretion (n=8, p=0.0002, p=0.005 and p=0.001, respectively). Conversely, treatment of aMSCs with the ROS scavenger N-acetyl-L-cysteine (NAC) reduced their IL-6, IL-8 and MCP-1 (n=6, p=0.03) production and improved their overall immunosuppressive capacity (n=6, p=0.03). In conclusion, these data suggest that altered mitochondrial function may serve as one of the core mechanisms by which aging alters MSC immunomodulatory capacity. Interventions aimed at restoring the mitochondrial function of aMSC may enhance their therapeutic efficiency.

T-1014

CELLULAR AND MECHANICAL MECHANISMS UNDERLYING REGENERATION IN DISTRACTION OSTEOGENESIS

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The skeleton can rapidly adapt to shifts in mechanical loading. Prolonged unloading - due to illness, immobilization, or microgravity - can result in increased resorption and high risk of fracture. Accumulated evidence indicates that mechanical cues, which include physical forces, alterations in extracellular matrix mechanics and changes in cell shape, are transmitted to the nucleus directly or indirectly to orchestrate transcriptional activities that are crucial for tissue regeneration. Although mechanotransduction is thought to occur via integration of multiple signaling pathways, the precise mechanism leading to downstream cellular responses is not well understood. We have developed a mouse model of mandibular distraction osteogenesis (DO) which allows for tracing of cell fate and genetic dissection of mechanotransduction during bone formation. Specifically, we examine cell-type-specific responses to mechanical force within distinct subpopulations of the mouse skeletal stem cell (mSSC) hierarchy. After determining that bone, cartilage, and stromal tissue are clonally derived in mice from lineage-restricted stem and progenitor cells in vivo, we employed this strategy to purify specific skeletogenic populations during mandibular distraction osteogenesis by prospective isolation using FACS. We show that mechanical force augments the numbers and function of multiple cell populations across the skeletal hierarchy, including mouse skeletal stem and progenitor cells and their differentiated subsets. We employed the assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) to profile open chromatin landscapes in these cell populations to understand the epigenetic changes in response to distraction. Mechanistically, distraction induces robust cell-matrix interactions that are coupled to cell-specific transcriptional responses via epigenomic pathways and pharmacological inactivation of this pathway disrupts bone formation. We have further identified a factor that is capable of restoring the bone-forming responsiveness of skeletal stem and progenitor cells, independent of cell-matrix interactions.

T-1016

BASAL P53 EXPRESSION IS INDISPENSABLE FOR BONE MARROW-DERIVED MESENCHYMAL STEM CELL INTEGRITY

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Bone marrow resident mesenchymal stem cells (MSCs) serve as a functional component of the perivascular niche that regulates hematopoiesis. They also represent the main source of bone formed in adult bone marrow, and their bifurcation to osteoblast and adipocyte lineages plays a key role in skeletal homeostasis and aging. Although the tumor suppressor p53 also functions in bone organogenesis, homeostasis and neoplasia, its role in MSCs remains poorly described. Herein, we examined the normal physiological role of p53 in primary MSCs cultured under physiologic oxygen levels. Using knockout mice, gene silencing, and pharmacological inhibitors we show that p53 inactivation depletes intrinsic mitochondrial ROS levels, and suppresses mitochondrial ROS generation and PPARG gene and protein induction in response to adipogenic stimuli. It also downregulates expression of TWIST2, which normally restrains cellular differentiation to maintain wild type MSCs in a multipotent state. Mechanistically, loss of adipogenic potential skews MSCs toward an osteogenic fate, which is further potentiated by TWIST2 down regulation, resulting in highly augmented osteogenic differentiation. Furthermore, p53-/- MSCs were also defective in supporting hematopoiesis as measured in standard colony assays due to decreased secretion of various cytokines including CXCL12 and CSF1. In wild type MSCs, we also show that transient exposure to 21% oxygen upregulates p53 protein expression resulting in increased mitochondrial ROS production and enhanced adipogenic differentiation at the expense of osteogenesis, and that treatment of cells with FGF2 mitigates these effects by inducing TWIST2. Thus, basal levels of p53 are necessary to maintain multi-potent

state or 'stemness' of MSCs, and graded increases in p53 expression in response to changing oxygen levels modulates cell fate and survival decisions. Thus, due to the critical function of basal p53 in MSCs, our findings question the use of p53-null cell lines as MSC surrogates, and also implicate dysfunctional MSC responses in the pathophysiology of p53-related skeletal disorders.

T-1018

IDENTITY AND TENOGENESIS OF TENDON STEM/PROGENITOR CELLS

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The repair of injured tendons remains a formidable clinical challenge, due to our limited understanding of tendon stem cells and the regulation of tenogenesis. The lack of a specific marker and a subpopulation of TSPCs is in part due to the limited number of lineage markers and the effects of ensemble averaging in conventional transcriptome analysis on cell populations. In this study we performed single cell analysis to characterize the gene expression profiles of individual cells isolated from tendon tissue. Subsequently, the roles of nestin during tenogenesis and function of nestin in TSPCs were further investigated by knockdown assay and the monoclonal analysis. We identified a subpopulation of nestin+ cells was identified within the heterogeneous tendon cell population by single-cell quantitative gene analysis. Utilizing gene expression Omnibus datasets and immunofluorescence assays, it was found that nestin expression level was dramatically lower in human tendinopathy tissue and that it was activated at specific stages of tendon development. The knockdown of nestin expression in TSPCs suppressed their clonogenic capacity and caused the loss of spindle-like morphology, reducing the tenogenic potential of these cells. We further observed that nestin+ cells were located mainly within the epitenon and endotenon and represent a multipotent teno-lineage progenitor population. Additionally, the number of nestin+ cells was increased following tendon injury and accompanied the upregulation of tenogenic marker genes, thus indicating that endogenous nestin+ cells may contribute to tendon repair. Moreover, the isolated nestin+ colony-forming cells exhibited similar multi-differentiation potential into osteogenic, chondrogenic and adipogenic lineages, whilst displaying superior tenogenic capacity compared to nestin- colony-forming cells. Hence, these findings demonstrate the crucial roles of nestin in tenogenesis and the maintenance of TSPCs phenotype, during both development and repair. Additionally, the gene marker expression profile and physical location of TSPCs within tendon tissues were further characterized, which could facilitate the clinical application of these cells in tendon regeneration.

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T-1020

OPTIMIZATION OF THE EFFICIENT PURIFICATION METHOD OF THE SKELETAL MUSCLE STEM CELLS FROM THE HUMAN INDUCED PLURIPOTENT STEM CELL DIFFERENTIATION CULTURE

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Duchenne muscular dystrophy is the most severe and common type of muscular dystrophy. Since lack of Dystrophin protein causes this disease, engraftment of healthy muscle stem cells, satellite cells (SCs), as the source of Dystrophin, has been considered as a promising curative treatment. To obtain a large amount of skeletal muscle stem cells (SMSCs), we have established a skeletal muscle differentiation protocol from human induced pluripotent stem cells (hiPSCs) without transgene overexpression (Takayama et al, in preparation). At first, we used a Myf5-tdTomato reporter line and purified SMSCs as Myf5+ fraction. This fraction was engraftable and showed efficient muscle regeneration with the recovery of muscular function of the engrafted legs (Takayama et al, in preparation). For clinical application, however, such reporter line should be avoided for the safety of cell therapy. Therefore, we have been investigating for purifying SMSCs from differentiation culture of hiPSCs by using cell surface markers. To eliminate undesired cell population, CD57 has been used for the marker of neuronal lineages (Borchin et al. Stem Cell Reports, 2013). We separately engrafted CD57+ and CD57- fractions in 12 weeks-differentiated hiPSCs into tibialis anterior muscle of cardiotoxin-treated immunodeficient mouse. Four or eight weeks after transplantation, we performed histological analyses. Only the CD57+ cells formed tumor while the CD57- cells never formed tumor. Furthermore, many of tumors widely express CD57. Therefore, we confirmed that CD57 is a useful marker to exclude immature neural crest cells which have tumorigenisity. Next, to improve the yield ratio of engraftment, we searched markers for SMSCs. Since CD82 was identified

as a novel marker of human SCs (Uezumi et al. Stem Cell Reports, 2016), we isolated the CD57-/CD82+ fraction from differentiated hiPSCs, and engrafted the cells as described above. Four weeks after transplantation, we detected more human spectrin+ myofibers in the CD57-/CD82+ cell-engrafted muscle than in the CD57-/CD82- cell-engrafted muscle. This result indicates that CD82 is a useful marker to accumulate hiPSCs derived SMSCs. These results suggest that two important issues of cell therapy, safety and efficiency, can be overcome by using cell surface markers.

T-1022

ENHANCED WOUND HEALING PROCESS USING HUMAN TRAUMA DERIVED MESENCHYMAL PROGENITOR CELLS IN A RAT MUSCLE CRUSH INJURY MODEL

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Current military operations involve complex traumatic orthopaedic injuries which necessities a prolonged wound closure that includes ischemia-reperfusion (IR). This is now known to result in sub-optimal healing which leads to the formation of scar tissue (fibrosis) and consequently impacts functional recovery and poor patient outcome. Thus, patients with these injuries may benefit from a treatment option that can suppress formation of fibrosis and promote key endogenous mechanisms required for normal and functional tissue regeneration. IR is a feature of deficient oxygen supply followed blood flow restoration and it's impact on post-traumatic musculoskeletal tissues is unknown but as shown in cardiac and kidney tissues, likely contributes to tissue fibrosis. Clinical trials using autologous mesenchymal stem cells (MSCs) for several diseases have indicated that their pro-angiogenic and cyto-protective properties provide regenerative benefit to the damaged tissues. Our laboratory has identified a sub-population of mesenchymal progenitor cells (MPCs) isolated from traumatized human muscle tissue and show similarity to MSCs in multilineage differentiation and trophic function. Here, we investigated the role of MPCs in a rat open muscle crush model to assess functional recovery and fibrosis. Injury was performed on Rowett nude rats (n=48) by crushing the biceps femoris with a clamp. TGFβ1 was injected at the injury site plus either: PBS, MPCs, TGFβ3-antibody (ab), or TGFβ3-ab with MPCs and open field analysis performed at 2, 4, 7, 14, and 21 days. Serum and muscle samples were harvested for cytokine and histopathological analysis at 7 and 21 days post-surgery. Groups treated with MPCs showed increased distance traveled at day 2 but not sustained as both groups recovered over time with low levels of α-SMA, collagen type III and thrichome staining for the presence of fibrosis. Analysis of serum (7 day) using cytokine arrays (R&D), groups treated with MPCs wound healing occurred earlier than control by up-regulating VEGF, CCL5, and CX3CL-1 and down-regulating CXCL-7, for angiogenesis and recruiting immune cells to injury sites to help tissue regeneration. Over results demonstrate that trauma derived MPCs can enhance wound healing to regenerate muscle tissue and functional recovery.

CARDIAC TISSUE

T-1026

IDENTIFICATION OF NOVEL EMBRYONIC VASCULAR STEM-LIKE CELLS AND THEIR EFFECT ON CORONARY VASCULAR REPAIR

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Endothelial cells participate in the development of many organs, including the heart. Despite advances in our understanding of how cardiac coronary vasculature is formed in the embryo, translating this knowledge into protocols for coronary vasculature repair has proved difficult. We have observed that at E15.5-adult hearts all major coronary vasculature endothelium and sub-endothelial cells in transgenic mice expressing the green fluorescent protein (GFP) under a regulatory element of the Nestin gene are N-GFP+. When N-GFP+ cells were conditionally ablated, there is partial loss of colony forming unit-fibroblast (CFU-F) activity and concomitant defects in coronary vasculature with severe loss of both endothelial cells and pericytes in the developing heart. Long-term self-renewing CFU-Fs in the developing heart at E11.5-neonate were restricted to a Pdgfra+/N-GFP- cell population and showed trans-germ layer in vitro multipotency. By contrast Pdgfra+/N-GFP+ cells showed limited CFU-F self-renewal with only vascular-lineage in vitro differentiation. Freshly isolated E15.5 Pdgfra+/N-GFP-/CD31-/Pdgfrb- and Pdgfra+/N-GFP+/

CD31-/Pdgfrb- cells were far more adept at forming vessel like structures in subcutaneous matrigel-plug assays than Pdgfra-/N-GFP-/CD31-/Pdgfrb- cells. These vessel-like structures had luminal cells, which expressed the endothelial marker CD31 and were enveloped by cells that expressed the pericyte marker, Pdgfrb. Pdgfra-/N-GFP+/CD31-/Pdgfrb- cells also formed vessel-like structures in matrigel, but these structures possessed only CD31 expressing endothelial cells and lacked Pdgfrb expressing pericytes. Cre mediated lineage tracing studies demonstrated that these cells originated from MesP1 precursors and were not derived from Wnt1 (neural crest) or Sox1 (neural epithelial) cells. The ability of various cell populations to contribute to coronary neovascularisation is being tested in a mouse ischemia-reperfusion myocardial infarct model. Taken together, we have identified novel populations of cardiac vascular stem-like cells in the developing heart. Characterising the regenerative capacity of these cells and unlocking their molecular regulation will help develop strategies to improve tissue regeneration following myocardial infarction.

T-1028

ACTIVATED CARDIAC PROGENITORS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS WITH ENHANCED CAPACITY TO GENERATE MATURE CARDIOMYOCYTES IN CARDIAC MICROTISSUE

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Cardiomyocytes derived from human iPS cells have promise in disease modeling, drug discovery, and therapy, but the challenge remains to create mature and organized cardiomyocytes like those found in the native heart. While groups have increased the maturity of iPS-derived cardiomycytes in extended culture with electrical, metabolic, and biomechanical stimulation of differentiated cardiomyocytes, we hypothesized that early epigenetic activation during the formation of cardiac progenitors could enhance their capacity to form mature cells. In a screen for nontoxic epigenetic activators, we found that administration of the innate immune agonist PolyIC during the formation of cardiac progenitors in defined small molecule monolayer differentiation increased histone acetylation and decreased HDAC expression and DNA methylation without increasing cell death. To test if epigenetic activation could restore signaling factors present in development, we measured the expression of endogenous Wnt ligands and inhibitors in cardiac differentiation lacking the small molecule inhibitor of Wnt and observed that PolyIC rescued endogenous Wnt inhibition and cardiomyocyte differentiation by augmenting decreased expression of Wnt 3 ligand. Compared to normal cardiac progenitors, activated progenitors had enhanced proliferation, future cardiomyocyte yield, and a dominant increase in cell proliferation pathways in RNAseq. This was lead by genes such as Jagged1 of the Notch pathway expressed in the developing cardiac crescent or heart tube, and Notch inhibition blocked progenitor proliferation. Activated progenitors differentiated into more mature cardiomyocytes based on larger size, optical upstroke velocity, oxidative metabolism, and expression of markers of CM maturation including cTnI, cardiac actin, and αMHC, which were blocked by Notch inhibition. Singularized activated progenitors could self-assemble and differentiate into organized, synchronized, beating cardiomyocyte sheets containing a network of HCN4+ conduction system cells that were sensitive to the nodal current blocker ivrabradine in contrast to the poorly coupled cardiomyocytes differentiated from nonactivated progenitors. Impact: Activated cardiac progenitors with the capacity to give rise to cardiomyocytes with enhanced maturation and organization hold promise for improving disease modeling, drug screening and cell therapy.

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T-1030

IPSC-DERIVED CARDIOMYOCYTES FOR PHENOTYPE-BASED, HIGH-THROUGHPUT DIAGNOSTIC TESTING FOR CHANNELOPATHY BY USING LQT

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Long QT syndrome (LQTS) is caused by hereditary cardiac channelopathies characterized by prolonged QT interval on an electrocardiogram. LQTS may precipitate malignant arrhythmia, resulting in syncope and sudden death. Genetic testing is currently utilized to assist treatment selection and prognostication with limited success. The predicted phenotype of the genetic abnormality often does not match the clinical phenotype due to low penetrance and variable expressivity of the syndrome. Therefore, development of phenotype-based, high-throughput diagnostic testing is necessary to identify clinically relevant genetic abnormalities. Here, we aim use LQTS patient-specific induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPSC-CMs) as a diagnostic platform for high-throughput in vitro phenotype screening. iPSCs were established from a LQTS type 2 (LQT2) patient with the KCNH2 mutation of A422T (1264G>A) causing loss of function of IKr associated with the trafficking defect of hERG. The differentiated CMs were analyzed on multi-electrode

array (MEA) to evaluate field potential duration (FPD) and its prolongation rate (ΔFPD) under administration of the IKr blocker, E4031. Fridericias' formula was used to standardize beat rate-associated dispersed FPD (FPDc). FPDc of LQT2A422T iPSC-CMs was significantly longer than that of control iPSC-CMs (144.6±1.99 vs. 254.7±25.8; p = 0.005). The effect of E4031 (100nM) on ΔFPDc was smaller in LQT2A422T iPSC-CMs than in control iPSC-CMs (50.3±5.6% vs. 105.7±22.1%; p=0.030), suggesting less IKr contribution to CM repolarization in LQT2A422T and consistent with an IKr disorder. Correction of the genetic abnormality in LQT2A422T-iPSCs (LQT2corr) using the CRISPR/Cas9 system shortened FPDc and normalized ΔFPDc by MEA. Additionally, IKr current density normalized as assessed by patch clamp. These results suggest that this IKr blocking protocol by MEA predicts IKr deficiency in LQT2 iPSC-CMs. This strategy may enable evaluation of IKr deficiency more accurately than genetic testing alone and to avoid ethical problems arising from revealing individual genes. Lastly, this strategy may be applicable not only to LQT2 but also to the other subtypes of LQTS and other cardiac channelopathies as a high-throughput screening assay.

T-1032

USE OF NOVEL ANTI-INFLAMMATORY DRUG-CONDITIONED HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES TO TREAT MYOCARDIAL INFARCTION

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Cardiovascular disease remains the leading cause of death worldwide. Due to the limited ability of cardiomyocytes to regenerate, their significant loss can lead to lethal consequences. Induced pluripotent stem cells (iPSC) may offer a great potential for cardiac regenerative therapy. However, a critical barrier to stem cell therapy is overcoming the robust inflammatory responses arising during tissue injury. The cytochrome P450 products, epoxyeicosatrienoic acids (EETs) are anti-inflammatory metabolites of arachidonic acid with cardioprotective effects, however, EETs are rapidly metabolized by the enzyme soluble epoxide hydrolase (sEH). Our team has developed novel inhibitors of sEH (sEHIs) to prevent the catalysis of EETs, thereby enhancing their cardioprotective activity. Indeed, we were the first to demonstrate the beneficial effects of sEHIs in clinically relevant models of cardiac hypertrophy, atrial fibrillation, and heart failure. To test the hypothesis that the survival of transplanted cells in the injured myocardium can be improved by reducing the inflammation, hiPSC-derived cardiomyocytes (hiPSC-CMs) were transplanted into mouse myocardium post myocardial infarction (MI) and treated with sEHIs. Multidisciplinary techniques were used to assess the survival and engraftment of hiPSC-CMs. In vivo bioluminescence imaging demonstrated that treatment with sEHI resulted in a significant increase in the survival and retention of transplanted hiPSC-CMs compared to cell treatment alone. Functional analysis showed a significant improvement in the fractional shortening by echo and MRI (61±2%) in the sEHI treated compared to the non-treated mice (54±1%). In vivo hemodynamic monitoring showed a significant decrease in the adverse remodeling with sEHI treatment. Single-cell based flow cytometric assays showed a decrease in apoptotic hiPSC-CMs and a decrease in reactive oxygen species production in the sEHI treated mice. sEHI-conditioned hiPSC-CMs showed a significant decrease in the activation of ERK1/2 and the reversal of the down-regulation of the transient outward K+ current compared to non-conditioned cells. Our findings suggest that suppression of inflammation and resolution of pre-existing fibrosis using sEHIs represents a potential and promising adjuvant to cardiac cell therapy.

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T-1034

FLUORESCENT HUMAN IPS REPORTER LINES OFFER USEFUL PLATFORMS TO STUDY CARDIAC SUBTYPE SPECIFICATION AND THEIR APPLICATIONS IN CELL-BASED THERAPIES OF CARDIAC DISEASE

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In order to use human iPS cells in the fields of regenerative medicine and drug development, it is crucial to identify functionally distinct cardiac subtypes and to study their functional aspects in healthy and diseased conditions. We use fluorescent proteins in human iPS cells for generating the cardiac subtype specific reporter lines: HCN4 ion channel and MLC2v myosin light chain are used as specific visualizing markers for nodal pacemaker cells and ventricular cardiomyocytes, respectively. Since HCN4 expression also can define cardiac progenitors in first heart field, the dual reporter lines contribute to

understanding of molecular mechanisms during cardiac differentiation. First, hiPSCs were introduced with human HCN4-eGFP-BAC (Bacterial artificial chromosome) semi-knock-in vector, in which the part of HCN4 exon1 was replaced with eGFP. Then, mCherry reporter gene is inserted at MLC2v locus through CRISPR/Cas9 genome editing. In this dual cardiac reporter hiPSCs, the expression of eGFP was first observed in early cardiac spheroids along with onset of self-contraction around day 10 of differentiation and was followed by mCherry expression after one month of differentiation. Isolated HCN4-eGFP-positive (HCN4+) cells expressed endogenous HCN4 and exhibited action potential characteristic of a nodal-phenotype including If current. Using imaging techniques, we demonstrated that HCN4+ cells established electrical coupling with HL-1 atrial CMs. These results demonstrated the potential of hiPSC-derived HCN4+ cells to act as a rate-responsive biological pacemaker. Isolated HCN4+ cells at early stage of differentiation gave rise to mCherry+ cells in prolonged culture. Currently, the properties of mCherry+ cells are under investigation. In addition, using HCN4-GFP-BAC line, we have made new fluorescent reporter lines, in which mCherry reporter gene is inserted at islet1 locus through CRISPR/Cas9. Since islet1 is the maker gene of second heart field, new dual reporter lines could help us to identify functionally distinct cardiac progenitor cells in vitro and to study underlying mechanisms during cardiac development.

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

T-1038

THE RNA BINDING PROTEIN QUAKING IS A KEY REGULATOR OF ENDOTHELIAL CELL DIFFERENTIATION, NEOVASCULARIZATION AND ANGIOGENESIS THROUGH DIRECT BINDING OF THE 3'UTR OF STAT3

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Regenerative therapy towards the repair and regeneration of cells and tissues in a clinical application is immensely promising but still faces many hurdles. One of the major obstacles is the reduced availability of suitable cells needed for therapeutic purposes. The capability to derive endothelial cells (ECs) from induced Pluripotent Stem (iPS) cells holds huge therapeutic potential for cardiovascular disease, which is the leading cause of death worldwide, and is associated with the appearance of endothelial dysfunction, a key event contributing to the formation and progression of atherosclerotic plaques. This study elucidates the precise role of the RNA-binding protein Quaking isoform 5 (QKI-5) during EC differentiation from both mouse and human iPS cells and dissects how RNA-binding proteins can improve differentiation efficiency towards cell therapy for important vascular diseases. iPS cells represent an attractive cellular approach for regenerative medicine today since they can be used to generate patient-specific therapeutic cells towards autologous cell therapy. In this study, using the model of iPS cells differentiation towards ECs, the QKI-5 was found to be an important regulator of STAT3 stabilisation and VEGFR2 activation during the EC differentiation process. QKI-5 was induced during EC differentiation, resulting in stabilisation of STAT3 expression and modulation of VEGFR2 transcriptional activation as well as VEGF secretion through direct binding to the 3' UTR of STAT3. Importantly, iPS-ECs overexpressing QKI-5 significantly improved angiogenesis and neovascularization and blood flow recovery in experimental hind limb ischemia. Notably, human iPS cells overexpressing QKI-5, induced angiogenesis on Matrigel plug assays in vivo only seven days after subcutaneous injection in SCID mice. These results highlight a clear functional benefit of QKI-5 in neovascularization, blood flow recovery and angiogenesis. They, thus, provide support to the growing consensus that elucidation of the molecular mechanisms underlying EC differentiation will ultimately advance stem cell regenerative therapy and eventually make the treatment of cardiovascular disease a reality.

Funding Source: BHF, BBSRC

T-1040

PRECONDITIONING OF OUTGROWTH ENDOTHELIAL CELLS WITH NATURAL PRODUCT COCKTAIL IS A NOVEL STRATEGY FOR DEVELOPMENT OF THERAPEUTIC STEM CELLS FOR ISCHEMIC DISEASES

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Although Outgrowth Endothelial Cells (OECs) play a pivotal role in vascular repair in ischemic tissues, the limitation on quality and quantity of the cells from patients is hindrance for therapeutic uses of autologous OECs. To overcome this, we investigated the potential of functional factor-based cocktail components for enhancement of cell functionalities. In this study, 3F (Fucoidan, Tauroursodeoxycholic acid, Oleuropein) preconditioning of OECs increased the proliferation

of the cells via ERK signaling pathways maintaining the expression of functional OEC surface markers. 3F also stimulate the in vitro tube formation capacity and promoted migration of OECs. To our knowledge, the findings of our study demonstrated that 3F preconditioning enhances the neovasculogenic potential of OECs by increasing their proliferation, survival, and endothelial differentiation. Pretreatment of OECs with 3F may thus provide a novel strategy for development of therapeutic OECs for severe ischemic diseases.

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HEMATOPOIESIS/IMMUNOLOGY

T-1042

DEREGULATED NF-KB SIGNALS AFFECT HEMATOPOIETIC STEM CELL QUIESCENCE, SELF-RENEWAL AND FUNCTIONS

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Identifying functions of specific signaling pathways in Hematopoietic stem cells (HSCs) may lead to new treatment strategies and therapeutic interventions for various hematologic disorders. Recent studies, including our own, identified that both loss and gain of functions of NF-κB in HSCs lead to pathologic hematopoiesis. Despite its significance, molecular mechanisms through which NF-κB influences HSC physiology remain largely unknown. Of note, constitutive activation of NF-κB is one of the key drivers of many hematologic malignancies; including AML, AEL and high risk MDS. These links to human disease provide a compelling rationale for further investigations into molecular mechanisms through which NF-κB regulates hematopoiesis, particularly HSCs. To this end, we followed a gain of function approach, and genetically engineered mice to constitutively express the activated form of IKK2 protein, a positive regulator of NF-κB, in HSCs. Using this model system, we show that constitutive activation of NF-κB in HSCs results in anemia, pancytopenia, bone marrow failure and premature death. Analysis of bone marrow from these mice revealed a striking loss of HSC pool, augmented proliferation and perturbed quiescence of HSCs, and lack of radioprotection by HSCs. Mechanistic studies involving Global gene expression profiling and Gene Set Enrichment Analysis (GSEA) indicated downregulation of several 'HSC signature' genes and up regulation of 'lineage signature' genes in HSCs with increased NF-κB. Interestingly, constitutive activation of NF-κB is sufficient to alter the regulatory circuits of transcription factors (TFs) that are critical to HSC self-renewal and functions. Furthermore, increased NF-κB activity in HSCs caused poor responses to thrombopoietin (TPO) mediated signal transduction, due to diminished expression of its receptor- Mpl, and therefore led to reduced levels of Cyclin dependent kinase inhibitor (CDKI) p57kip2, a key regulator of HSC quiescence. Promoter analysis and Chromatin Immunoprecipitation (ChIP) studies indicated augmented binding of NF-κB to the regulatory sites of key transcription factors that are essential for lineage differentiation. At this annual meeting, we would be discussing the molecular mechanisms and targets through which NF-κB affects HSC biology.

T-1044

AUTOPHAGY MAINTAINS THE METABOLISM AND FUNCTION OF YOUNG AND OLD HEMATOPOIETIC STEM CELLS

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With age, hematopoietic stem cells (HSCs) lose their ability to produce all blood cells, resulting in a decline in immune responses and increased rates of blood diseases in the elderly, and autophagy has long been linked to aging and longevity. To understand how autophagy regulates HSC function, we generated mice with conditional deletion of the essential autophagy gene Atg12 (Atg12cKO) in the blood system. We observed premature aging phenotypes in Atg12cKO HSCs that resemble old HSCs, including similar differentially methylated regions, enhanced myeloid differentiation, rapid loss of self-renewal potential and HSC depletion under regenerative challenges such as transplantation. Analyses of Atg12cKO HSCs also revealed an excess of mitochondria, resulting in increased OXPHOS, ROS production, cell cycle activation, and precocious myeloid differentiation. Analyses of aged GFP-LC3 autophagy-reporter mice revealed a striking heterogeneity in old HSCs, with ~70% having low autophagy levels but ~30% having high autophagy levels, regulated by mTOR activity. Old HSCs with low autophagy levels were strikingly similar to autophagy-deficient young HSCs, with metabolic over-activation and functional exhaustion after transplantation, while old HSCs with high levels of autophagy resembled young healthy HSCs and displayed robust long-term regenerative capacity. Furthermore, we have now found similar results in a different system of stromal stem cells. Our results demonstrate that HSCs require autophagy to maintain a low metabolic, quiescent state that preserves their regenerative capacity, and that during aging HSCs become increasingly dependent on autophagy activation for their functional maintenance. Old HSCs

with low autophagy levels are severely impaired, likely causing age-associated declines in regenerative potential and blood production. Our data suggest that increasing autophagy in old HSCs could help promote HSC fitness and rejuvenate the aging blood system, which we are now testing using known longevity-promoting and autophagy-activating interventions. Furthermore, utilizing this functional separation of old HSCs based on autophagy levels and RNAseq, we have now identified additional key drivers of functional aging in HSCs.

T-1046

OVEREXPRESSION OF LIN28A IN HEMATOPOIETIC STEM CELLS INDUCES SYSTEMIC METABOLIC ALTERATIONS IN TRANSPLANTED RECIPIENTS

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LIN28A and LIN28B are highly-conserved RNA-binding proteins important in development and disease. We recently showed that lentivirus-mediated overexpression of Lin28a or its downstream target, Hmga2, in adult mouse hematopoietic stem cells (HSCs) activates a fetal-like heightened self-renewal activity in their progeny regenerating in transplanted recipients. In these experiments, we noted that recipients of Lin28a-overexpressing cells gained weight more rapidly than controls. To quantify and elucidate the mechanism of this systemic "metabolic" effect, we monitored a series of mice transplanted with the equivalent of 8% of the bone marrow (BM) cells of a single mouse after pooling BM harvests from multiple primary mice transplanted 6 weeks before with small numbers of highly purified (CD45+EPCR+CD48-CD150+; ESLAM) Lin28-overexpressing, Hmga2-overexpressing or control-transduced HSCs. 8 weeks post-transplant, all secondary mice were transferred to a high fat diet and their weights monitored. Recipients of Lin28-overexpressing BM demonstrated a significantly higher percentage weight gain at the 4 week time-point as compared to control mice (9.7 versus 3.6; $p < 0.01$). As Lin28a has been shown to regulate glucose metabolism, we also compared fasting blood glucose levels in the same 2 groups of mice. Recipients of Lin28-overexpressing cells showed significantly lower fasting glucose levels compared to controls (5.23±0.28 versus 5.95±0.17 mM, $p < 0.05$). Nevertheless, glucose tolerance was only marginally different and significant only at the 30 minute time-point ($p=0.02$). Secondary recipients of the progeny of Hmga2-overexpressing HSCs showed a less marked difference in percentage weight gain (7.4, $p=0.02$, at the 4 week time-point), and no difference in fasting glucose or glucose tolerance parameters. This study provides evidence for a new role of Lin28a on systemic metabolism mediated through a HSC transplant-mediated mechanism.

T-1048

LIN28B-LET-7-CBX2 REGULATES DEVELOPMENTAL MATURATION OF HEMATOPOIETIC PROGENITORS

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Maturation of tissues occurs in synchrony with the developmental demands of the host. One such illustration, the hematopoietic system, matures over time from the embryo to adult on schedule with other organ systems. We have previously shown that oligopotent common myeloid progenitors (CMPs) in the midgestation fetal liver (FL) are biased toward robust erythrocytic output supportive of rapid intrauterine prenatal growth, while postnatal bone marrow (BM) CMPs produce predominantly myeloid cells to provide innate immunity. We previously found that expression of the heterochronic regulator Lin28b in the FL specifies prenatal erythroid-dominant CMP output by repression of the let-7 family of microRNAs that drive pro-myeloid transcriptional programs. Recent studies indicate that the ultimate fate outcomes of CMPs may be pre-programmed by upstream lineage-biased multipotent progenitors (MPPs). Therefore, we hypothesized that remodeling of the heterogeneous MPP compartment might underlie developmental changes in myeloerythropoiesis. We found that during maturation from the midgestation FL to adult BM, erythroid-biased MPPs diminish in quantity and clonogenicity ($P = 0.02$), while myeloid-biased MPPs increase ($P < 0.0001$). Ectopic expression of LIN28B in adult BM resulted in remodeling of the MPP compartment, with a decrease in myeloid-biased MPPs ($P = 0.03$) and adoption of a FL-like MPP distribution. To begin to understand the mechanisms by which the MPP compartment undergoes normal maturation, and how Lin28b regulates this process, we used RNA sequencing of hematopoietic progenitors. We identified differentially expressed transcripts between FL, BM, and LIN28B-expressing BM progenitors. We used gene set enrichment analysis against the gene regulatory subnetworks constructed in our CellNet computational platform to identify the key developmentally regulated subnetworks modulated by LIN28B. Within these subnetworks, we found the let-7 target mRNA Cbx2, which encodes a component of Polycomb repressor 1 and which is highly expressed in FL and LIN28B-expressing BM progenitors relative to normal BM

progenitors. We found that ectopic expression of Cbx2 in BM hematopoietic stem cells represses myeloid differentiation. These studies identify a novel axis regulating maturation of myeloerythropoiesis.

T-1050

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) DEFICIENCY IMPAIRS HEMATOPOIETIC STEM CELL SELF-RENEWAL AND REGENERATION VIA DECREASED DNA REPAIR

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Deficiencies in DNA repair mechanisms impair hematopoietic stem cell (HSC) self-renewal with age (Rossi et al. Nature 447:725-729,2007). Epidermal growth factor receptor (EGFR) signaling promotes DNA repair in somatic cells via activation of DNA PKcs. We recently reported that HSCs express EGFR and that EGF promotes HSC regeneration following irradiation via repression of PUMA (Doan et al. Nat Med 19:295-304,2013). Here, we sought to determine whether cell-specific inhibition of EGFR in HSCs would disrupt HSC function in homeostasis or following myelosuppression. Inducible expression of dominant negative EGFR (EGFR-DN) in stem cell leukemia (SCL)+ hematopoietic stem/progenitor cells (HSPCs) in adult mice (SCL-tTA; tetO-EGFR-tr) did not alter baseline complete blood counts, BM cell counts or HSPC numbers. However, competitive repopulation assays demonstrated that mice transplanted with BM cells from adult EGFR-DN mice displayed markedly decreased engraftment of donor CD45.2+ hematopoietic cells compared to mice transplanted with BM cells from EGFR-wild type mice (63.1% vs. 0.2% CD45.2+ cell engraftment at 8 weeks post-transplant, $p < 0.0001$). At day +7 following exposure to 500 cGy total body radiation (TBI), EGFR-DN mice displayed significantly decreased BM ckit+sca-1+lin- (KSL) cells ($p=0.02$), SLAM+KSL cells ($p=0.005$), CFCs ($p=0.002$) and significantly decreased survival at day +30 compared to EGFR wild type mice (15.4% survival vs. 50.0% survival, $p=0.04$). BM cells isolated from EGFR-DN mice at day +7 following 500 cGy TBI displayed a severe repopulating defect compared to BM cells from irradiated, EGFR-wild type donor mice following competitive repopulation assay (19.4% vs. 0.01% CD45.2+ cell engraftment at 16 weeks post-transplant, $p=0.0003$). Mechanistically, BM KSL cells from EGFR-DN mice displayed increased γ-H2AX levels at baseline and following 100 cGy irradiation, coupled with a decrease in p-DNA PKcs compared to EGFR-wild type KSL cells ($p=0.003$, $p < 0.0001$ and $p=0.005$). These results suggest that EGFR deficiency impairs DNA repair in HSCs, which contributes to a defect in HSC self-renewal capacity at baseline and following irradiation. These findings provide new insights into the role of EGFR-mediated DNA repair mechanisms in regulating HSC self-renewal capacity.

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T-1052

SLC6A15 MAINTAINS HEMATOPOIETIC STEM CELLS BY REGULATING AMINO ACID HOMEOSTASIS

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Specific amino acid metabolism has been shown to regulate the pluripotent state of mouse embryonic stem cells; however, whether specific amino acid transporters (AATs) are involved in stem cell maintenance is unknown. Here, using RNA sequencing to screen the gene expression profiles of 16 different hematopoietic cells including hematopoietic stem, progenitor cells, and mature lineage cells, among 45 AATs tested, we identified amino acid transporter Slc6a15 as a hematopoietic stem cell (HSC) specific AAT. Inhibition of SLC6A15 with loratadine led to decreased HSC number and functionality, suggesting the amino acid (AA) substrates of Slc6a15 might be required for HSC maintenance. Indeed, individual deprivation of each essential AA (EAA) showed that withdrawal of Valine (Val), Methionine (Met), or Threonine (Thr), which are substrates of Slc6a15, caused a > 90% decline of HSCs. Furthermore, by applying tRNA aminoacylation assay, we demonstrated that loratadine inhibition of Slc6a15 impaired the efficiency of Val and Thr transport at the expense of HSC maintenance. Mechanistically, SLC6A15 inhibition elicited the AA starvation response pathway, resulting in the phosphorylation of eIF2α (p-eIF2α) and inhibition of eIF2α-dependent general translation. p-eIF2α in turn induced translation of c-Myc, which caused HSC activation, differentiation, and apoptosis. Loss of c-Myc expression rescued the Val, Met, and Thr deprivation-mediated HSC reduction in ex vivo culture. Thus, Slc6a15 maintains HSCs through operating as a sensor and transporter of specific AAs required by HSCs.

T-1054

T REGULATORY CELLS FROM IPSC AS A TOOL TO MODULATE IMMUNITY AND TOLERANCE

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Organ transplantation is a life saving therapy for thousands of patients every year. Inducing immunological tolerance in transplant recipients promises to increase the longevity and improve the quality of life these patients experience due to the side effects and failures of broad-spectrum immunosuppression. T regulatory (T reg) cells have emerged as a safe and potentially potent cellular immunotherapy for tolerance induction in transplantation. However, current T reg isolation and ex-vivo expansion protocols have struggled to produce large enough numbers and may be impossible in certain deceased donor settings. Induced pluripotent stem cell (iPSC)-derived T regs would offer a scalable, renewable, and tailorable solution. Previous reports of iPSC-derived T cells have been limited by a xenogenic (murine) feeder cell layer expressing a murine notch 1 ligand and the immature state (CD4/CD8 double positive) of the resulting cells. Here we report a comparison between murine (OP9) and human (thymic fibroblast) feeder cells expressing human MHC class II and the human notch 1 ligand DLL4 in the production of mature CD4 SP cells and their potential to differentiate into T reg cells.

Funding Source: Department of General Surgery at Boston University School of Medicine

T-1056

A MOLECULAR AND CELLULAR BASELINE FOR FETAL TO ADULT HEMATOPOIESIS IN THE HUMAN

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Hematopoietic stem cells (HSCs) can self-renewal and generate daughter cells to maintain and replenish all the blood system making them a prime target for regenerative therapy. Last year, ~20 000 patients in the US received hematopoietic stem cell therapy (HCT); however, donor tissues and protocols vary widely (i.e. bone marrow (BM), mobilized peripheral blood (mPB), umbilical cord blood (CB), etc). Notably, as little as 0.1% of transplanted material represents long-term HSCs. Consequently, the remaining cellular constituents, non-HSC, has a deep impact on the HCT efficacy and adverse events (i.e. GVHD or BM failure). Additionally, the proliferative and differentiation potential of HSCs change in a tissue-specific manner. Despite these profound changes in cell properties and composition little is known about the factors and mechanisms regulating variation in these tissues. Thus, there is an urgent need to understand the cellular components of HCT tissues to better customize therapy and minimize side effects. We have previously used single cell mass cytometry (CyTOF) to map many simultaneous features in healthy human hematopoietic system, delineating both new cell identities and regulatory relationships. Here, we leverage this technique to identify molecular and cellular differences that arise during normal human hematopoietic development. We simultaneously captured 40+ features including phenotypic cell identity and known hematopoietic transcription factors, such as GFI1, GATA2, BMI1, etc. at single cell level on different human hematopoietic tissues, including CB, FL, BM, and mPB. For the first time, we have determined cellular landmarks that are highly conserved across all tissues that can be used as a reference map to compare human hematopoietic cells not only from diverse organs, but also from different treatments and culture conditions. As a consequence, we were able to identify small cellular variances between fetal (FL and CB) and adult (BM and mPB) hematopoietic tissues. More importantly, our results pinpoint the coordination of key molecular regulators governing cell states that HSCs endure during development. Overall, we provide new insight into the complex process of human immune development, and a reference framework for ex vivo manipulation strategies for HCT customization.

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T-1058

CELL-BASED THERAPY OF AIRWAY INFECTIONS UTILIZING HUMAN IPSC-DERIVED MACROPHAGES PRODUCED IN BIOREACTORS

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Hematopoietic differentiation of human induced pluripotent stem cells (hiPSC) opens new opportunities for innovative cell-based treatment strategies. However, clinical translation is currently hampered by a lack of therapeutically relevant quantities of functional effector cells. To overcome this hurdle, we here demonstrate the efficient production of iPSC-derived mature hematopoietic cells in fully-equipped stirred tank bioreactors and their subsequent therapeutic application as a novel cell-based treatment approach targeting acute respiratory infections. First, we established a suspension-based hematopoietic differentiation protocol able to continuously generate human iPSC-derived macrophages, granulocytes and erythrocytes from "myeloid cell forming complex (MCFC)" intermediates, harboring CD34+/CD45+ hematopoietic progenitor cells. Upscaling of the process to a stirred tank bioreactor in 120ml scale resulted in the continuous generation of 20-30 million iPSC-derived macrophages (iPSC-Mac) per week for up to five weeks. Produced iPSC-Mac displayed a highly pure CD45+CD11b+CD14+CD163+TRA-1-60- surface phenotype and a transcriptional profile similar to peripheral blood derived phagocytes. Moreover, iPSC-Mac efficiently phagocytosed Pseudomonas aeruginosa in vitro, secreted important pro-inflammatory cytokines such as IL2, IL6, IL8, MCP1, TNFa, and IFNa2 and up-regulated defined gene sets associated with activated innate immunity and pathogen clearance. Of note, pulmonary iPSC-Mac transplantation (PiMT) rescued immunodeficient mice from established respiratory P. aeruginosa infection as demonstrated by significantly reduced disease scores (1.8±0.2 vs. 8.1±0.2), restored activity, as well as normal body temperature (35.4±0.5 vs. 29.4±1.7°C) and lung function. Improved disease parameters were in line with a profound reduction in lung bacterial burden (2x10^8±1x10^8 vs 2x10^4±0.6x10^4 CFU) and normal lung histology in PiMT treated animals compared to solely infected mice. In summary, we demonstrate for the first time the continuous production of hiPSC-derived myeloid cells in industry-compatible bioreactors and introduce a novel and antibiotic independent cell-based treatment strategy for respiratory infections.

T-1060

METFORMIN INDUCES FETAL HEMOGLOBIN IN HEMATOPOIETIC STEM AND PROGENITOR CELLS FROM PATIENTS WITH SICKLE CELL ANEMIA

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Elevated fetal hemoglobin (HbF, alpha2gamma2) ameliorates sickle cell disease (SCD) by inhibiting polymerization of HbS; one drug, hydroxyurea, can induce HbF in about 50% of individuals with SCD. We identified FOXO3 as a positive regulator of gamma globin through analysis of rare variants from whole exome sequence data from SCD patients. We then manipulated FOXO3 expression in normal human CD34+ hematopoietic stem and progenitor cells (HSPCs) induced to undergo erythroid differentiation and examined the effects on HbF production. Transfection of CD34+ HSPCs with lentivirus expressing FOXO3 shRNA that reduced levels of the corresponding RNA by 80% reduced gamma globin mRNA by 60% compared to control cultures expressing scrambled shRNA (n= 3 separate experiments, $p < 0.0005$). Metformin, an FDA-approved drug used for type 2 diabetes, is known to increase FOXO3 expression and therefore, we tested

its effects on HbF expression in CD34+ HSPC-derived erythroblasts. Metformin treatment caused a dose dependent increase in % HbF, as measured by HPLC. HPLC analysis at d14 showed that %HbF (HbF/(HbF + HbA) rose from 10.3% at baseline, to 19.8% and 30% at 50 and 100 μM metformin (n= 3, p=0.04 and 0.005). For comparison patients on standard metformin therapy for diabetes typically exhibit plasma drug levels of 70 μM. When cultures were treated with metformin (100μM) and HU (30μM), %HbF was increased to 67% compared to 26% and 30% in cultures treated with HU or metformin respectively as single agents (n=3, p= < 0.0001, p=.0016, and p=0.005). Thus, HU and metformin show additive effects, on HbF induction, indicating that combination therapy may be more effective than standard therapy of HU alone. In addition, metformin may be a superior agent than HU as single therapy because it does not cause myelosuppression, and therefore requires minimal laboratory monitoring, and does not arrest erythropoiesis, making it a potentially effective agent for quantitative beta hemoglobinopathies. Taken together, our results indicate that FOXO3 is a positive regulator of gamma globin expression and a potential therapeutic target for HbF induction. A pilot clinical trial of metformin, alone or combined with hydroxyurea, has been approved by the Baylor College of Medicine IRB.

PANCREAS, LIVER, KIDNEY

T-1062

MODELLING MITCHELL-RILEY SYNDROME IN VITRO USING IPS CELLS DERIVED FROM A RFX6 MUTANT PATIENT

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The forkhead transcription factor Regulatory Factor X, 6 (RFX6) is essential for the development of the mammalian pancreas. In humans, RFX6 mutations are responsible for Mitchell-Riley Syndrome (MRS), which is characterized by neonatal diabetes with pancreatic hypoplasia. Similarly, Rfx6 mutant mice fail to develop all islet endocrine cell types and die soon after birth. Although RFX6 mutations yield a dramatic disease phenotype, our knowledge about RFX6 function and regulation is extremely limited. To address this deficit, we have generated human induced pluripotent cell (hiPSC) lines from a Syrian MRS patient with a novel RFX6 null mutation. Based on published, but limited work in the mouse, our expectation was that RFX6 functions specifically at the transition between NGN3+ pancreatic endocrine progenitors to mature hormone-containing islet subtypes. Our data, however; show that (1) during directed differentiation toward the pancreatic lineage, RFX6 expression precedes the activation of the master regulator gene PDX1, with weak RFX6 expression first detected as early as day 4; (2) MRS iPSC fail to activate the pan-endodermal organ marker SOX9 as well as PDX1; and (3) wild-type H9 human ES cells (hESC) routinely produce >85% PDX1+;NKX6-1+ pancreatic progenitors by day 12 of differentiation, whilst MRS iPSC consistently yield < 30%. Taken together, we propose that RFX6 plays a previously unanticipated, earlier role in human pancreatic development and that RFX6 expression is bi-phasic, with increasing levels during primitive gut tube development followed by a decline and then resolving to mature beta cells. RFX6 binds and activates the INSULIN promoter in the human beta cell line EndoC-βH2 (Chandra et al., 2014 Cell Reports) and thus, like PDX1, may also function during beta cell homeostasis. Lastly, both humans and mice display developmental defects in the gall bladder and duodenum, suggesting that RFX6 function is not limited to the pancreatic lineage.

T-1064

ALVEOLAR TYPE 2 CELLS PROLIFERATION AND DIFFERENTIATION POTENTIALS IN EMPHYSEMA MODEL MICE

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Emphysema is characterized by irreversible destruction of alveolar structure accompanied by decrease of alveolar type 2 (AT2) cells and an increase of apoptotic cells, although the role of AT2 cells is not fully understood. A variety of site-specific stem cells play a key role in repairing and maintaining lung tissues, and AT2 cells have been known as a stem cells providing AT1 cells in alveolar walls. We hypothesized that the function of AT2 cells were disturbed in emphysematous lungs, and that might be involved in the emphysema progression. To examine the proliferation and differentiation potentials of AT2 cells, we utilized the two different emphysema models in mice; elastase- and long-term cigarette smoke (CS)-induced models. Surfactant protein C (Sftpc)/GFP (CBA/Ca x C57BL6J) mice were either intratracheally instilled with elastase (5 U in 100 ul) or exposed to mainstream CS through the nose 5 days/week for 3 to 6 months. At sacrifice, lung epithelial cells were collected, analyzed and sorted using flow cytometry based on their GFP and EpCAM expression. Sorted lung epithelial cells were co-cultured with lung fibroblasts collected from C57BL/6 naïve mice in our standardized "in vitro colony forming assay (CFA)". The number of growing GFP+ (i.e. AT2 stem cell) colonies were quantified under the microscope. EpCAM+/GFP+ cells harvested from

elastase-induced emphysema lungs formed significantly fewer colonies compared to control mice (89.3±23.4SE vs. 396.0±47.2/well, $p < 0.05$). In contrast, EpCAM+/GFP+ cells harvested from CS-induced emphysema lungs formed significantly more colonies compared to control mice (229.3±22.0SE vs. 108.0±17.4/well, $p < 0.05$). Although most of the GFP+ cells are positive for aquaporin 5 (an AT1 cell marker) in control lungs, GFP+ cells in harvested from CS-induced emphysema lungs are less likely to express aquaporin 5 (65.4% vs. 86.3%). In summary, the proliferation of AT2 cells is impaired in the elastase-induced emphysema, but is rather enhanced in the CS-induced emphysema in mice. Also AT2 cells in CS model mice have less potential to differentiate to AT1 cells. This result implies the diverse function of AT2 stem cells in the two emphysema models.

T-1066

GENERATION AND CHARACTERIZATION OF NKX6.1-GFP REPORTER LINES IN A HUMAN INDUCED PLURIPOTENT STEM CELL BACKGROUND FOR ISLET CELL RESEARCH

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Human pluripotent stem cells (hPSC) can be differentiated into physiologically-relevant cell types for disease modelling, drug screening and cell replacement therapy. In the diabetes field, much attention has been directed towards developing protocols for deriving insulin producing beta cells of the pancreatic islet as these are either partly or completely deficient in various forms of diabetes. Directed differentiation protocols allows for the generation of insulin producing beta-like cells that express many markers characteristic of bona fide beta cells. However, the differentiation efficiency varies across hPSC lines and it is not clear how well the hPSC-derived beta-like cells compare to beta cells with regards to gene expression and functionality. Thus, more efforts should be directed towards refinement of differentiation protocols and development of research tools for the purification and characterization of the hPSC-derived beta-like cells. To this end we have tagged the endogenous NKX6.1 locus of a human induced pluripotent stem cell (hiPSC) derived from a healthy individual with a T2A-GFP sequence. NKX6.1 was selected as it is expressed in pancreatic progenitors during development and subsequently becomes restricted to beta cells. Sequencing confirmed correct insertion of the T2A-GFP reporter construct in multiple, clonal hiPSC lines, with differentiation of the NKX6.1-GFP hiPSC clones to pancreatic progenitors yielding GFP positive cells. An almost complete overlap between GFP and NKX6.1 positive cells was demonstrated by fluorescence microscopy and flow cytometry, confirming the faithfulness of the reporter construct. NKX6.1-GFP hiPSC lines were differentiated through sequential developmental stages to yield GFP and C-peptide co-expressing beta-like cells capable of insulin secretion. FACS isolation revealed that the GFP-positive cells were enriched for expression of NKX6.1 and other beta-cell specific genes compared to unsorted and GFP-negative cell populations. Finally, we demonstrate that the cell populations can be cultured following isolation and used for functional studies. These results demonstrate the development of NKX6.1-GFP hiPSC reporter lines and the utility of such lines for improving studies of hPSC-derived beta-like cells.

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T-1068

TRANSCRIPTIONAL ANALYSIS OF HUMAN PLURIPOTENT STEM CELL-DERIVED HEPATOCYTES AND COMPARISON TO HUMAN PRIMARY HEPATOCYTES IN SUSPENSION AND 2D CULTURE

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Hepatocytes derived from human pluripotent stem cells (hPSC) have a great potential in replacing primary human hepatocytes (PHH) as in vitro tools for drug development, toxicity assessment, metabolism studies etc. PHH suffer from several disadvantages, such as low availability, donor-to-donor variations, and a rapid loss of key metabolizing and transporter functions in conventional 2D cultures. Until recently, the hPSC-derived hepatocytes have shown deficiencies in functionality that have hampered the successful use of these cells for some applications. However, recent improvements in the differentiation protocols have allowed for the generation of highly homogenous hPSC-derived hepatocyte cultures that are approaching their primary counterpart in CYP enzyme activities and gene

expression profiles. In this study we used microarray technology to investigate the transcriptional profile of hPSC-derived hepatocytes and compared it against the expression profiles of PHH from three donors. Hepatocytes derived from hPSC were cultured for 13 days post-thawing and harvested for subsequent total RNA extraction. In parallel, cryopreserved PHH from three donors were thawed and plated according to standard procedures, and total RNA was extracted either prior to plating of the cells or after 24 hours in 2D culture. Global expression analysis showed that the hPSC-derived hepatocytes expressed many important hepatocyte genes in levels comparable to PHH. Also, a clear effect of the plating and the 2D culture was observed in the PHH. More than 2,000 genes that showed at least a two-fold difference in expression after 24 hours in culture were identified. Interestingly, the hPSC-derived hepatocytes showed a higher resemblance to the 24 hour-cultured PHH than to PHH prior to plating, which suggests that the hPSC-derived hepatocytes exhibit similarities to a "plated hepatocyte" phenotype. By identifying key genes that were changed during 2D culturing of PHH, possible targets may be detected that could help to counteract the "plated" phenotype in the hPSC-derived hepatocytes.

T-1070

CRYOPRESERVATION OF ENCAPSULATED STEM CELL DERIVED PANCREATIC BETA-CELL PROGENITORS FOR THE TREATMENT OF DIABETES

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Worldwide there are close to 382 million people suffering from diabetes. All patients with Type 1 diabetes and 25% of patients with Type 2 diabetes are treated with exogenous insulin. However, insulin injections lack the minute-to-minute precision islets provide and can lead to debilitating complications. Islet transplantation, an alternative treatment approach, has been limited by a shortage of donor organ tissue and the need for lifelong immunosuppression. Human embryonic stem cells (hESCs) are considered a potential solution to the problem of supply and encapsulation inside a robust retrievable device (Theracyte) could mitigate the need for immunosuppression. Our lab has previously demonstrated that encapsulated human embryonic stem cell derived islet progenitor (hESC-IP) cells mature and acquire glucose responsiveness following transplantation, curing diabetes in mice. The encapsulation devices were immunoprotective, prevented cell escape, and could be transplanted subcutaneously. The ability to cryopreserve hESC-IPs pre-loaded into devices could enhance quality control, consistency and dissemination of the therapy to clinical trial sites and eventually to treatment centers. Unfortunately, traditional slow freezing approaches have not worked well for cells inside durable devices. We hypothesized that this was due to formation of ice crystals that damage cells during slow freezing. To overcome this, our lab has focused on an alternative freezing approach called vitrification in which cells are frozen at high speed to achieve a glass state and avoid glass crystals. We have identified vitrificant solutions that are tolerated by hESC-IPs and engineered specialized technology for hyperfast cooling of encapsulated cells in liquid nitrogen. In a proof of principle study, we have tested the hyperfast cooling device, successfully vitrifying encapsulated cells that were thawed three weeks later. The cryopreservation of hESC-IPs inside immunoprotective devices could speed the development and ultimate use of encapsulated stem cell treatments for diabetes. Moreover, the technology is amenable to use with other encapsulated stem cell derived treatments.

Funding Source: CIRM

T-1072

LIVER PROTECTIVE EFFECT OF RESVERATROL IN DIABETIC RATS RECEIVING AUTOLOGOUS TRANSPLANTATION OF ADIPOSE-DERIVED STEM CELLS

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Liver dysfunction is one of complications regarding diabetes. Stem cell transplantation and supplementation of herbal medicine are potential therapeutic strategies in the treatment of liver dysfunction induced by diabetes. This study aims to investigate if synergistical effect can be observed for liver protection in diabetic rats receiving both of adipose-derived stem cell (ADSC) transplantation and resveratrol supplementation. Experimental animals were divided into four groups including sham, DM, DM+ADSC (DM rats with ADSC) and DM+R-ADSC (DM rats with ADSC preconditioned with resveratrol). Compared to sham group, all liver markers including GOT and GPT were increasing in DM group. In addition, activation of fibrosis, suppression of survival and activation of apoptosis pathways were observed in liver tissues in DM group. By contrast, all markers were improved in DM rats with ADSC (DM+ADSC)

when compared to DM group. Furthermore, significant improvement for liver markers was observed in DM+R-ADSC group when compared to DM. These results illustrate that precondition of ADSC with resveratrol increases ADSC capability in the treatment of liver dysfunction induced by diabetes.

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EPITHELIAL TISSUES

T-1074

MICRORNA-21 MEDIATES THE PROTECTIVE EFFECTS OF MESENCHYMAL STEM CELLS DERIVED FROM IPSCS TO HUMAN BRONCHIAL EPITHELIAL CELL INJURY UNDER HYPOXIA

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The airway epithelial cell injury is a key triggering event to activate the allergic airway inflammation such as asthma. We previously reported that administration of MSCs significantly alleviated allergic inflammation in mouse model of asthma and mmu-miR-21/ACVR2A axis may be involved in it. However, whether mesenchymal stem cells (MSCs) protect bronchial epithelial cells injured by hypoxia and the underlying mechanism is still unknown. In our study, the human bronchial epithelial cells of BEAS-2B were induced to apoptosis with a hypoxia mimic of CoCl2. We found that the treatment of MSCs derived from induced pluripotent stem cells (iPSCs) significantly decreased the apoptosis of BEAS-2B cells. There was high miR-21 expression in the injured BEAS-2B cells after the treatment of MSCs. Transfection of miR-21 mimic significantly decreased the apoptosis of BEAS-2B, and transfection of miR-21 inhibitor significantly increased the apoptosis. More importantly, the protective effects of MSCs on injured BEAS-2B was reversed by transfection of miR-21 inhibitor. We further identified that CoCl2 stimulation increased ACVR2A expression in both mRNA and protein levels. Moreover, transfection of miR-21 mimic further up-regulated ACVR2A expression induced by CoCl2 and transfection of miR-21 inhibitor down-regulated ACVR2A expression. In addition,the apoptosis between ACVR2A positive and negative cells were different. Almost all the apoptosis were occurred in ACVR2A positive cells.Our data suggest that MSCs protect bronchial epithelial cell from hypoxia injury via miR-21 and ACVR2A may be an important target involved in it. It provides the possible wide application of MSCs on the epithelial cells under hypoxia.

T-1076

MTORC1 INHIBITS B-CATENIN ACTIVITY AND INTESTINAL EPITHELIAL CELLS PROLIFERATION DURING INFLAMMATION

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The intestinal epithelium is highly dynamic and actively turned over. Intestinal epithelial cells (IECs) form a selectively permeable barrier that separates luminal contents and the underlying tissue compartments. Epithelial barrier properties are maintained during proliferation-migration and ultimate shedding of epithelial cells. In intestinal epithelium β-catenin signaling is critical for maintenance of epithelial homeostasis and for tissue regeneration during physiological and pathological conditions. During inflammation, homeostatic properties of the epithelium are compromised, and it is well appreciated that deleterious effects of inflammation on epithelial homeostasis are mediated in part by TNF-α and IFN-γ However, the mechanisms controlling β-catenin transactivation in inflamed tissues are incompletely understood. The proinflammatory cytokine IFN-γ influences IECs homeostasis in a biphasic manner by acutely stimulating proliferation that is followed by a sustained decrease of this process in spite of continued mucosal injury. IFN-γ activates phosphoinositide-3-kinase (PI3K) in a variety of cell lines, and PI3K regulates proliferation, cell growth, and differentiation of the IECs. PI3K through its downstream target, Akt, enhances β-catenin-mediated cell proliferation in epithelial cells. Thus, PI3K signaling pathway appears as a good candidate to control β-catenin signaling in IEC during IFN-γ-mediated inflammation. Our current studies suggest that PI3K/Akt signaling inhibits β-catenin-mediated cell proliferation and disrupts barrier function during inflammation by activating its downstream target mTORC1. In a model of colitis we have reported that paradoxically, during inflammation Akt activation reduces β-catenin co-transcriptional activity, decreases β-catenin-mediated cell proliferation and ultimately triggers apoptosis. Now, our preliminary results suggested that inhibition of β-catenin function during inflammation is mediated by mTORC1, another downstream target of Akt; however, the regulatory machinery controlling this process is unknown. Taken together, these results suggest that mTORC1 could inhibit β-catenin mediated cell proliferation in colonic epithelium.

T-1078

REGULATION OF INTESTINAL STEM CELL DIFFERENTIATION AND EPITHELIAL MONOLAYER HEALTH: CREATING A REPRESENTATIVE IN VITRO INTESTINAL MODEL

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Differentiation of LGR5+ stem cells into one of the many epithelial subtypes relies on a complex cocktail of cues from the surrounding microenvironment. The crypt-villus structural organization of the intestine, with stem cells lying at the bases of intestinal crypts, allows differential activation of Wnt, BMP, and Notch pathways as transamplifying cells migrate upwards and secure their differentiated fate. Each of the epithelial subtypes, including enterocytes, goblet, enteroendocrine cells, and paneth cells, are vital to intestinal health; thus regulation of these cues are important for proper intestinal function. Recent advances have allowed the in vitro culture of intestinal stem cells as organoids, relying on Wnt3A, Noggin, and R-spondin-3 for stem cell renewal. Differentiation of these stem cells into an epithelial monolayer typically occurs in the presence of these molecules, with additional factors added to inhibit Notch or Wnt pathways and force differentiation to desired states. While providing a means of obtaining a mixed monolayer, this method is not well-controlled or representative of the native intestinal niche. In this study, we utilized a transwell system to investigate the regulation of intestinal stem cell differentiation by both intestinal myofibroblasts and the enteric nervous system (ENS), with the hope that native cell cues would induce differentiation into all epithelial subtypes. Dissociated stem cells were seeded on collagen coated transwells apically to myofibroblasts or mixed cultures of primary enteric neurons and glia. After 24 hours of differentiation, transepithelial resistance (TEER) measurements for cultures with myofibroblasts were 2x greater than control or ENS cultures. TEER remains roughly 2x higher than controls on day 7, with upregulation of zona occludens 1, which occurs only when given Wnt3A in media. Myofibroblasts increase the longevity of cultures, with cells surviving to 3-4 days longer than lone epithelial cultures, suggesting their role in epithelial maintenance. Fluorescent images show Muc2 expression, indicating goblet cells, for myofibroblast cocultures. No significant changes were seen in the presence of ENS cultures, but further analysis will investigate differential protein expression in the presence of both the ENS and myofibroblasts.

T-1080

EPITHELIAL STEM CELLS ARE FORMED BY SMALL-PARTICLES RELEASED FROM PARTICLE-PRODUCING CELLS

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Recent spatiotemporal report demonstrated that epidermal stem cells have equal potential to divide or differentiate, with no asymmetric cell division observed. Therefore, how epithelial stem cells maintain lifelong stem-cell support still needs to be elucidated. In mouse blood and bone marrow, we found a group of large cells stained strongly to eosin and contained coiled-tubing-like structures. Many of them were tightly attached to each other to form into large cellular clumps. After sectioning, we found that these large cell-clumps composed of not cells but numerous small particles, however with few small “naked” nuclei. The small particles were about 2 to 3 μm in diameter and stained dense red with eosin, which suggest that they are rich in proteins. Except the clumps cmposed of small particles, we also identified clumps formed by fusion of the small particles, and clumps of newly formed nucleated cells. These observations suggested that these small particles further fused and underwent cellularization. E-cadherin was expressed on particle-fusion areas, some “naked” nuclei and on the newly formed nucleated cells, which suggested that these particles can form into epithelial cells via fusion and nuclear remodeling. In addition, we observed similar-particle fusion before epithelial cellularization in in vivo mouse kidney-duct after kidney ischemia, which suggests that these particles can be released in the blood and carried to the target tissues for epithelial-cell regeneration. Cells that mainly located in the center of the cellular clumps were rich in proteins and expressed Oct4 and E-cadherin, which suggested that they had become tissue-specific epithelial stem cells. Our data provide evidence that these large particle-producing cells are the origin of epithelial stem cells. The epithelial stem cells are newly formed by particle fusion.

T-1082

ANALYSIS OF GENES IN COLONIC LGR5+ STEM CELLS SPECIFICALLY ACTIVATED AT LOW-DOSE-RATE RADIATION EXPOSURE

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We previously found that ionizing radiation did not induce isometric effects on colonic Lgr5$^+$ stem cell in mice when those animals irradiated with the same dose but different dose-rate. High-dose-rate radiation induced the replenishment of colonic Lgr5$^+$ stem-cell pool, whereas low-dose-rate radiation did not. To identify what happened in colonic Lgr5$^+$ stem-cell pool under the different dose-rate conditions, colonic Lgr5$^+$ stem cells were harvested from the mice two weeks after 1 Gy of high-dose-rate (30 Gy/h) or low-dose-rate (3 mGy/h) irradiation by cell sorting and were analyzed gene expression profiles by RNA-Seq. We found that pathways related to DNA damage responses, cell growth, cell differentiation, and cell death were upregulated in high-dose-rate irradiated Lgr5$^+$ stem cells. On the other hand, pathways related to apical junctions and extracellular signaling were upregulated in low-dose-rate irradiated colonic Lgr5$^+$ stem cells. Interestingly, it is well known that biological events through apical junctions play an important role in exclusions of transformed cells, when they surrounded by normal epithelial cells, as a "cell competition". Therefore, we speculated that the cell competition through apical junctions and extracellular ligands might contribute to the "dose-rate effects" in Lgr5$^+$ cell replenishment. In order to understand the mechanism, we focused on 69 genes significantly upregulated in low-dose-rate irradiated group and called them DREDGE (Dose-Rate Effect Determining GEnes). For example, the expression of *Dredge1* gene showed 4.6 and 4.3 times higher than that in non-irradiated and high-dose-rate irradiated groups, respectively, and expresses on apical part of cells. Based on these findings, we would like to suggest a possible mechanism of dose-rate effect on colonic stem cells.

T-1084

USE OF HOMEOBOX GENE EXPRESSION PATTERNS TO DETERMINE ANATOMICAL REGIONS OF ORIGIN FOR BODY SURFACE TISSUES DERIVED FROM ADULT MICE

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Anatomical regions of the skin have distinct functions and anatomical characteristics, including thicker or thinner epidermis, more or fewer hair follicles, and lighter or darker skin. For a better therapeutic outcome of skin transplantation, site-specific characteristics of grafted tissues need to be taken into account in terms of their functionality and beauty. However, there is no method for evaluating positional information of epidermal cells. Homeobox genes have a highly conserved homeobox domain, are expressed along the anterior–posterior axis, and direct the body plan in the animal development process. Although expression of several HOX genes is known to be retained as the positional information in adult tissue, their expression patterns in the body surface tissues in adult mammals are still incompletely understood. In this study, we investigated the expression patterns of 40 homeobox genes, including 39 Hox genes and the paired box 6 (Pax6) gene, in body surface tissues of adult mice. Thirty-three of the 39 Hox genes as well as Pax6 were expressed in a site-specific manner and categorized into four groups in accordance with the region, posterior, distal, dorsal, and other, where they tended to be expressed. Based on the results obtained, we proposed, for the first time, a method for determining anatomical regions of origin for body surface tissues derived from adult mice using Hox genes and Pax6. Evaluation of expression levels of at least seven Hox genes and Pax6 should be sufficient to distinguish 11 anatomical body surface tissues derived from the adult mouse body. The proposed method may be useful not only for determining the origin of surface tissues from specific anatomical regions of the mammalian body but also for predicting positional information of epithelial cells generated from pluripotent stem cells.

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T-1086

MOUSE SALIVARY GLAND-DERIVED MYOEPITHELIAL CELLS SHOWS STEM/ PROGENITOR CELL PROPERTIES IN VITRO

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Salivary hypofunction caused by Sjögren syndrome or radiation therapy for head and neck cancer can lead to not only disruption of oral homeostasis but also systemic-pathological event such as aspiration pneumonia in severe cases. Several salivary gland stem/ progenitor cells have been identified and known to be involved in salivary gland development, homeostasis, and regeneration. However, the precise cell derivation of salivary gland stem/progenitor cells have not fully understood. Here, we identified stem/progenitor cells in salivary gland by detecting slow-cycling cells, which is one of stem cell features. Briefly, BrdU was intraperitoneal injection at 2-days-old mice twice in a day for 3 days. Label-retaining cells (LRCs) were detected immunohistochemically. LRCs in SMG were found in a part of epithelium, including duct-, acinar-, and myoepithelial-cells after 10 weeks. Localization of LRCs were more specifically in spindle cells like as myoepthelial cells located between luminal and stroma cells after 30 weeks. Therefore, myoepithelial cells was expected to be putative stem cells and isolated by flow cytometry using by cell surface markers EpCAM and CD49f. The data from RNA-seq showed CD49fhigh subset contained αSMA and Myh11 positive cells, a marker of myoepithelial cells, and also CK14, CK5 were expressed at the same levels as in CD49fmid subset, suggesting that CD49fhigh subset is enrichment of myoepithelial cells. The selection of a CD49fhigh subset showed enhanced sphere formation ability compared to that of CD49fmid and CD49flow. Interestingly, CD49fhigh subset-derived sphere had ability to differentiate into ductal lineage in vitro. In conclusion, myoepithelial cells are one of slow cycling cells in SMG. Furthermore, these cells were enriched in CD49fhigh subset and showed multipotent properties by sphere formation. Therefore, enriched myoepithelial cell compartment may contain stem/progenitor cells in salivary glands.

STEM CELL NICHES

T-1088

EFFECTS OF VEGF ON PERIODONTAL LIGAMENT MESENCHYMAL STEM CELLS

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The periodontal ligament (PDL) is a connective tissue providing tooth stability and nutrition. There is a pool of mesenchymal stem cells within human PDL that might serve as a promising source for cell-based treatment of various pathologies. However, existing strategies are restricted by low cell survival and engraftment; therefore we investigate the effect of various active molecules on stem cell properties. Vascular endothelial growth factor (VEGF) is one of the growth factors with highest biologic activity. Our research reveals that VEGF is able to induce the differentiation of PDL stem cells into endothelial-like cells after short term treatment. The growth factor can also promote cell proliferation after a period of 48h and induce mineralization nodules formation in the cell culture after three weeks incubation. Alkaline phosphatase activity in the cell culture was also significantly increased after 1 week cultivation, while total collagen production was found to be decreased. The VEGF concentration applied for our in vitro experiments is 50 ng/ml. Based on our results, we suggest that VEGF has positive effects on cell proliferation and differentiation into various cell types in vitro depending on the treatment duration and the additional components of cell culture medium. Our results provide new options for engineering of artificial tissues based on autologous mesenchymal stem cells and growth factors activity.

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T-1090

MYELIN BASIC PROTEIN EXHIBITS A NICHE-DEPENDENT REGULATION OF REGIONALLY DISTINCT NEURAL STEM CELLS IN THE BRAIN AND SPINAL CORD OF THE ADULT MOUSE

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Myelin basic protein (MBP) is a major protein constituent of the myelin sheath expressed by oligodendrocytes of the central nervous system (CNS). Its canonical function is to enable the adhesion of multilayered compact myelin throughout the CNS for improved saltatory conduction. We have recently demonstrated that MBP inhibits proliferation of two distinct populations of spinal cord derived neural stem cells (NSCs), in a dose dependent fashion at 25, 50, and 100ug/mL – without affecting their survival. Interestingly, this inhibitory effect of MBP is regionally distinct along the neuraxis as revealed by the finding that primary cultures of forebrain-derived NPCs are not inhibited by exogenous MBP at similar concentrations unless it is presented in the context of the spinal cord niche. The effects of MBP is seen when NSCs from Shiverer mice (devoid of mature MBP) are co-cultured with periventricular spinal cord tissue from control mice (MBP-expressing). The numbers of Shiverer derived spinal cord neurospheres is significantly reduced (up to 86%). No decrease in neurosphere formation was observed when Shiverer brain NSCs were co-cultured with control (MBP-expressing) brain periventricular tissue, hinting that brain and spinal cord MBP differ in their ability to inhibit neurosphere formation. This regionally distinct responsiveness is completely lost in cultures with pure populations of neural stem and progenitor cells (i.e. cells derived from passaged neurospheres – grown in the absence of niche cells). Indeed, pure populations of NPCs are not inhibited by exogenous MBP, irrespective of where they were derived (i.e. spinal cord or forebrain). The finding that MBP's effect on NPCs is regionally distinct along the neuraxis is consistent with MBP being classified as an intrinsically unstructured protein whereby its structure and function are both dependent on the microenvironment it is presented in, further supporting a role for the stem cell niche in regulating MBP's inhibitory actions on NPCs. These findings have implications for the finding of potential therapeutic targets for stem cell based regenerative strategies for brain and spinal cord repair.

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T-1092

ANALYSIS OF THE DIFFERENTIATION CAPACITY OF ADULT STEM/PROGENITOR CELLS IN THE PARENCHYMAL-NICHE OF THE RODENT PITUITARY GLAND

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The anterior lobe of the pituitary gland is a master endocrine tissue composed of five types of endocrine-cells and non-endocrine-cells. Among non-endocrine-cells, Sox2-expressing stem/progenitor cells play a role in the regeneration of endocrine-cells in the adult pituitary. Studies of the rodent pituitary gland have demonstrated that pituitary SOX2-positive stem/progenitor cells constitute sub-populations defined by expression of calcium binding protein, S100β. However, little is known about alteration of character of SOX2-positive cells based on the existence of S100β. Recently, we succeeded to isolate parenchymal-stem/progenitor cell niche, termed PS-cluster, by stepwise protease treatment of the adult pituitary of S100β/GFP-TG rats. PS-clusters were classified based on the contents of S100β-expressing cells (GFP-clusters and null-GFP-clusters), and only GFP-clusters showed the capacity to differentiate into pituitary hormone-producing cells by 3D-culture method. In this study, we aimed to further analyze the differentiation capacity of stem/progenitor cells in the GFP- and null-GFP-clusters by 2D-culture method. On 2D-cultivation, the proliferation activity of GFP-clusters was higher than that of null-GFP-ones. In addition, immunostaining for SOX2 demonstrated that GFP-clusters, but not null-GFP-clusters, gave raise to SOX2-negative cells after 7-day cultivation. Immunostaining for pituitary hormones and commitment cell markers demonstrated that GFP-clusters were negative for these pituitary lineage cell markers. Therefore, to analyze differentiation of GFP-clusters into non-pituitary cell lineages, we performed immunostaining for Myogenin, which is a myoblast marker, and quantitative RT-PCR for Myogenin and Myoglobin. Immunostaining and qPCR demonstrated that only GFP-clusters had an ability to differentiate into skeletal muscle cells. Collectively, the present study demonstrated the different character of pituitary stem/progenitor cells in the parenchymal niche based on the

existence of S100β-expressing cells, and that S100β-expressing ones have an ability to differentiate into non-pituitary cell lineage in addition to pituitary hormone producing cells.

T-1094

REGULATING TENSION BETWEEN MATRIX AND CELL BY MAGNETIC NANOPARTICLE-BASED SYSTEM FOR OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

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Increased interest in mechanotransduction of stem cell has led researchers to propose various physical parameters on the controlling of stem cell fate. Still, there are many lurking factors in understanding how the cells "sense" and "respond to" the microenvironment. We hypothesized the microenvironment's parameters, represented as matrix stiffness, distance between cell-matrix adhesion points and porosity of matrix, share the key parameter, tension. Thus, we hypothesize that the tension, the stretching force, between extracellular-matrix and the stem cells is the main parameter for regulating stem cell fate. In this work, we induced magnetization of human mesenchymal stem cells (hMSCs) by incorporating magnetic nanoparticles (MNPs) into hMSCs. Magnetized hMSCs were exposed to the various strengths of external magnetic force. By doing so, we could regulate the intensity of matrix to cell tension. As a result, COL-1, OPN and OCN, the osteogenic marker genes, expressed significantly higher in the magnetized hMSCs group which was exposed to the magnetic force than those of the other groups, with no MNPs and/or magnetic force. Furthermore, we found the strength of the external magnetic force that hMSCs showed increased osteogenic differentiation induction. By this research, we could develop the defined method for controlling the fate of stem cell by regulating matrix to cell tension.

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T-1096

NEURAL STEM CELL NICHE: ROSETTE STRUCTURE AND FUNCTION

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Neural rosette formation is a critical morphogenetic process during our brain development, whereby neural stem cells are enclosed in rosette niches to equipoise proliferation and differentiation. How neural rosettes form and provide a regulatory micro-environment remains to be elucidated. We employed the human embryonic stem cell-based neural rosette system to investigate the structuring and function of neural rosettes. Our study showed that neural rosette formation consists of 5 types of cell movements, including intercalation, constriction, polarization, elongation and lumen formation. Ca2+ signalling plays a pivotal role in the five steps by regulating the actions of the cytoskeletal complexes, ACTIN, MYOSIN II, and TUBULIN in intercalation, constriction, and elongation, which in turn control polarizing elements, ZO-1, PARD3, and β-CATENIN in polarization and lumen formation during the course of neural rosettes formation. we further demonstrated that the dismantlement of neural rosettes mediated by the destruction of cytoskeletal elements promoted neurogenesis and gliogenesis prematurely, implicating an intake rosette structure is essential for orderly neural development.

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EYE AND RETINA

T-1100

ESTABLISHMENT OF PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS FOR DISEASE MODELING OF X-LINKED JUVENILE RETINOSCHISIS

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X-linked juvenile retinoschisis (XLRS) is an inherited retinal degenerative disease that affects males early in life. Patients with XLRS typically experience moderate visual loss and vitreous hemorrhage, though in some severe cases retinal detachment may occur. RS1 (also known as XLRS1) is the only gene known to causes XLRS disease. The gene product of RS1 is a secreted protein known as retinoschisin, whose reported function is to maintain the retina structure and cellular organization. Previous studies in mouse model have demonstrated that retinoschisin is highly expressed in photoreceptor and bipolar cells. However, the function and mechanisms of retinoschisin in human retina still unclear. To investigate the role of retinoschisin in human retina, we sequenced the RS1 gene from XLRS patients. Most of the observed mutation sites are in discoidin domain, which would inhibit secretion and encourage retainment in ER. Concurrently, we generate integration-free patient-specific iPSCs with c.625C>T mutation in RS1 gene to model XLRS in vitro. RS1c.625C>T and WT-

iPSCs were differentiated into 3D optic-vesicle like (OV) organoids. At the pluripotent stage and early stages of OV differentiate, we observed that RS1 mutation does not significantly influence the pluripotency markers nor neural retina progenitor cell marker expression. Our OV organoids then formed laminated neural retina tissue, and expressed various photoreceptor markers and RS1 after differentiated day 50. However, during photoreceptor maturation, RS1 mutant-OV organoids had splitting in laminated retinal layer, which was very similar to clinical phenotype in vivo. To further understand the impact of the discoidin domain mutation in RS1 gene, we examined the protein expression, secretion and intracellular localization of wild type and RS1c.625C>T in iPS-derived OV organoids and 293 cell line. Our results showed that diseased-linked c.625C>T mutation did affect protein expression in mature OV organoids. RNA-Seq results also suggested some molecular influence of RS1 mutant during retinal differentiation. In our study, we demonstrated that the establishment of integration-free patient-specific RS1c.625C>T-iPSC is a powerful tool for unveiling molecular events in XLRS, which could also finding treatment options in XLRS disease.

T-1102

CEP290 OR IFT88 LOSS OF FUNCTION IN RPE SUGGESTS A ROLE FOR PRIMARY CILIA IN HUMAN IPSC-RPE MATURATION AND PROVIDES INSIGHTS INTO CILIOPATHY-INDUCED RETINAL DEGENERATION

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Retinal pigment epithelium (RPE), a ciliated monolayer in the back of the eye, is critical for maintaining the health and integrity of adjacent photoreceptors. Our previous studies demonstrate that the primary cilia play important role in the regulation of RPE monolayer maturation. However, a direct genetic link between primary cilium proteins and RPE maturation has not been explored yet. The goal of this study is to determine the role of CEP290 and IFT88, two key primary cilium proteins in RPE development and maturation using iPSC-RPE from patients with CEP290 mutations and shRNA knockdown of IFT88. Healthy or CEP290 mutant human iPSC derived RPE (iPSC-RPE) were grown on semi-permeable transwells to generate a confluent monolayer. IFT88 knockdown was mediated using doxycycline-inducible shRNA lentivirus. Immunocytochemistry, scanning electron microscopy, gene expression, phagocytosis and electrophysiology were used to determine the level of iPSC-RPE monolayer polarization and maturation. CEP290 mutations led to abnormal primary cilium and apical processes in iPSC-RPE accompanied with largely reduced capability to phagocytose photoreceptor outer segments. Similarly, IFT88 knockdown prevented primary cilia formation and yielded sparse apical processes. Gene expression analysis demonstrated that the RPE-signature genes and adult-specific RPE genes were both downregulated in CEP290 mutant and IFT88 knockdown RPE cells. Electrophysiology recordings revealed that the suppression of primary cilia with IFT88 shRNA led to significantly decreased RPE transepithelial potential and whole tissue resistance; the electrical responses to physiological stimuli were also dramatically reduced, indicating deficient RPE maturation and functionality. Our results demonstrate that CEP290 mutations and IFT88 knockdown severely affected primary cilia formation and profoundly disrupted iPSC-RPE monolayer maturation. This study presents direct evidence for the critical role of primary cilia in RPE maturation, and provides insights into the mechanism of ciliopathy-induced retinal degeneration.

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T-1104

PLGA ENCAPSULATED FGF2 MICROBEADS ACTIVATE THE PROLIFERATION OF THE NORMALLY DORMANT ENDOGENOUS HUMAN RETINAL PIGMENT EPITHELIUM STEM CELLS

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The retinal pigment epithelium (RPE) is a monolayer of cells beneath the neural retina. It plays a critical role in supporting retinal function and normal vision, and RPE dysfunction is implicated in several eye diseases. As a central nervous system derivative, adult human RPE (hRPE) cells are relatively stable throughout life and have limited regenerative ability. However, a previous in vitro study demonstrated the existence of a subpopulation of self-renewing hRPE stem cells (hRPESCs) in adult human RPE obtained from donated globes. Thus, we hypothesized that RPESCs could be activated to proliferate within the human retina to promote endogenous RPE layer repair. To test our hypothesis, we prepared hRPE/choroid/sclera explants from aged human donors and used these as a 3D model that closely mimics the in vivo environment of hRPE cells. Fibroblast growth factor 2 (FGF2) (also known as basic FGF), which was previously found to stimulate the proliferation of hRPE cells, was encapsulated in PLGA-based microbeads to create a sustained formulation releasing FGF2 at approximately 10ng/ml (StemBeads FGF2). FGF2 beads were applied to the explant for 10 days and

activated a small population of hRPE cells to proliferate in the cultured explants from aged donors. Further, we demonstrated that RPESCs can be identified in the mouse, and that intravitreally injected FGF2 microbeads can stimulate endogenous proliferation in the RPE layer, which further supported our findings in human explants. In vitro single cell culture and clonal analysis indicates that RPESCs have increased expression of the receptor FGFR2 relative to the non-proliferative hRPE cells ($P < 0.05$). The discovery of endogenously activated hRPESCs and the specialized expression of FGFR2 on hRPESCs open up the possibility of developing a novel reparative therapy for RPE-related eye diseases such as age-related macular degeneration.

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T-1106

DIRECT REPROGRAMMING TO GENERATE PHOTORECEPTORS FOR TREATMENT OF PHOTORECEPTOR DEGENERATION

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The loss of photoreceptors is a leading cause of vision loss in degenerative eye diseases such as retinitis pigmentosa (RP), age-related macular degeneration and diabetic retinopathy. Currently there are few effective treatments for retinal diseases, in particular no treatment to regenerate lost photoreceptors. For instance, RP is an inherited retinal dystrophy due to progressive loss of photoreceptors, with a prevalence of ~1 in 4000 and affecting >1 million worldwide. An important feature in both patients and animal model of RP is that, despite the loss of photoreceptors, most of the inner retinal neuronal structure and visual pathway remain largely intact. Therefore, cell replacement therapy, such as photoreceptor transplantation, provides hope for RP patients to restore vision. Donor photoreceptors can be derived from stem cells, such as pluripotent stem cells or retinal/neural progenitors. Although photoreceptor transplantation represents a promising strategy to restore vision loss in RP, immune-rejection and integration of transplanted cells remain obstacles that need to be addressed. In recent years, direct reprogramming of one somatic cell type to another has been reported without passing through an intermediate stem cell state. This can be achieved using defined transcription factors, often master regulators, to manipulate the transcriptome to alter cell fate. Multiple studies have demonstrated the feasibility to reprogram glial cells into different types of neurons in vivo in the brain, spinal cord and retina. This study aims to test the feasibility of reprogramming retinal glial cells into photoreceptors. We adapted the CRISPRa system for multiplex expression of reprogramming factors. This allowed us to efficiently screen different combinations of transcription factors for reprogramming into photoreceptors. Development of direct reprogramming technology represents an innovative approach to generate photoreceptors, providing a step towards novel regenerative therapy to replace photoreceptor losses in retinal degeneration.

Funding Source: University of Melbourne Ophthalmology Trust Funding

NEURAL DEVELOPMENT AND REGENERATION

T-1108

DERIVATION OF PUTATIVE PORCINE NEURAL CREST STEM CELLS FROM INDUCED PLURIPOTENT STEM CELLS

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Prior to transplantation, preclinical study of safety and efficacy are needed in regenerative medicine. The pig (Sus scrofa) is increasingly being regarded as promising species of model animals that have similar neural anatomy and biology with humans. Here, we report the first derivation of putative porcine neural crest (NC) stem and progenitor cells from induced pluripotent stem cells (iPSCs) without coculture. The conversion of porcine iPSCs to neurectoderm and neural crest cells accomplished by blocking both BMP (LDN 193189) and Activin/Nodal/TGF β (SB431541) branches of SMAD signaling, a process termed dual SMAD inhibition (dSMADi). After in vitro expansion, neural rosettes gave rise to the enriched, NC surface marker, p75+ subfractions exhibiting self-renewal capacity with stem cell markers such as Nestin and CD133. Also, p75+ NC precursor cells propagated in vitro and generated neurospheres that capable of further differentiation showing distinctive morphology and phenotype in peripheral neurons (TUJ1+), glial cells (GFAP+) and mesenchymal lineages (VIMENTIN+). Although further studies will be needed to characterize its properties, the putative porcine NC cells derived in this study might provide an exciting tool to bridge the present gaps in neuroscience studies between rodents and humans.

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T-1110

IDENTIFICATION OF A POPULATION OF DORMANT NEURAL PROGENITOR CELLS IN THE ADULT MOUSE DENTATE GYRUS

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New neurons that are generated in the adult mammalian hippocampus are thought to arise from multipotent neural stem cells in the subgranular zone of the dentate gyrus. Radial glia-like cells (RGLs), which share many traits with developmental radial glia cells, have previously been identified as the resident neural stem cells in the adult mouse dentate gyrus. Though initially thought to be a homogeneous population, in recent years it has become apparent that there is significant heterogeneity within the RGL population. Here we identify a very dormant population of RGLs using a Hopx-CreERT2::YFP mouse line. We performed clonal analysis of individual RGLs using a low dose of tamoxifen to sparsely label Hopx-expressing RGLs (referred to as Hopx-labeled RGLs). We find that the Hopx-labeled RGLs are quiescent for long periods of time, but when they become activated they predominantly generate neurons and virtually no astrocytes. Interestingly, once they are activated, they rapidly generate neurons and deplete. These results suggest that continuous neurogenesis in the adult hippocampus originates not just from multipotent, self-renewing RGLs, but also from dormant neurogenic progenitor RGLs. Identification of this specific population using the Hopx-CreERT2 mouse line offers a tool to understanding its properties, regulatory mechanisms and functional contribution to brain function under physiological and pathological conditions.

T-1112

INVESTIGATION OF NEUROGENIC DEFECTS IN FRAGILE X SYNDROME

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Fragile X Syndrome (FXS) is the leading monogenic cause of intellectual disability and autism spectrum disorder. It is caused by an expansion of a trinucleotide repeat in the 5'UTR of the Fragile X Mental Retardation-1 (FMR1) gene. We have previously reported that induced pluripotent stem cells (iPSCs) derived from FXS patients exhibit reduced neurogenesis and genome-wide differences in DNA methylation and gene expression compared to controls (Brain, 2017). In the current study, we have found that the impaired neurogenic competence in FXS cells is likely due to flawed establishment of dorsal telencephalic identity, which is manifest by low expression of the dorsal forebrain gene FOXG1 and abnormal neural rosette formation and neuroepithelial polarity. Our gene expression analysis suggests dysregulation in the FXS cells of Notch, WNT, and TGFβ signaling pathways that are essential for dorsal fate specification in the telencephalon. We are dissecting the roles of signaling pathways underlying the neurogenic phenotype using genomic rescue experiments and assessing the phenotypic outcomes with gene expression, DNA methylation, and electrophysiological analyses. We are developing assays to screen small molecule candidates for their ability to rescue or reduce the impaired patterning and neurogenesis in the FXS cells. Our long-term goal is to develop a phenotypic assay that is amenable to high-throughput therapeutic drug screening.

T-1114

MOUSE INDUCED NEURAL STEM CELL TRANSPLANTS DECREASED BEHAVIORAL DEFICITS AND ATTENUATED NEUROPATHOLOGICAL CHANGES IN THE YAC128 MOUSE MODEL OF HUNTINGTON'S DISEASE

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There is neither a cure for Huntington's disease (HD) nor an effective treatment. However, several research approaches are aimed at developing new treatments for HD, including those that are focused on replacing neurons. Although stem cell therapy is one of the most promising strategies for neuronal replacement, the safety and efficacy of this approach needs to be optimized. The purpose of this study is to test the efficacy of intra-striatal transplantation of induced neural stem cells (iNSCs) that are derived from induced pluripotent stem cells (iPSCs) for treating HD. For this purpose, we developed mouse adenovirus-generated iPSCs, differentiated them into neural stem cells in vitro, labeled them with Hoechst, and then transplanted them bilaterally into striata of 10-month old male and female wild type (WT) and HD

YAC128 mice. We tested the ability of these transplanted iNSCs to survive, differentiate into neuronal phenotypes, and reduce motor deficits of YAC128 by testing them on an accelerating rotarod task at one day prior to transplantation, and then weekly for 10 weeks. Our results showed an amelioration of locomotor deficits for YAC128 mice that received iNSC transplantation. Following testing, the mice were sacrificed, and their brains were analyzed using immunohistochemistry or Western blot (WB). The results from our histological examinations revealed that iNSCs had survived and showed evidence of differentiation into region-specific neurons in both WT and HD mice, as evidenced by co-labelling of Hoechst-labelled transplanted cells with NeuN and DARPP-32. There was no evidence of tumors and our preliminary WB data suggests an increase in DARPP-32 and BDNF receptors (TrkB) in striata of the YAC128 mice that received iNSC transplantation. Further analyses for detecting possible markers of inflammation and mutant huntingtin aggregate formation is still ongoing. Collectively, our data suggest that iNSCs may provide a safe and effective option for neuronal replacement therapy.

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T-1116

FATE CHOICES BY HUMAN NEURAL STEM CELLS

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During development cells have to make several choices of fate, for example deciding to maintain their identity or choose between several other fates. We have studied the fate choices human neural stem cells take in vitro when induced to differentiation by removal of growth factors. Our observations suggests that neural stem cells from individuals with psychiatric and neurodevelopmental diseases seem to have difficulty to take similar fate decisions as neural stem cells of healthy individuals. Functional analyses of the neural stem cell differentiation show delayed or accelerated differentiation in patient specific cells of different disease, respectively. This indicate a de-regulated fate choice between staying as a stem cell or leave and go into differentiation by the patient cells compared to healthy cells. Further, once a neural stem cell decided to differentiate it has to decide what cell type to become, in this case a neuron or a glia. Our in vitro neural stem cell experiments show a skewed neuron to glia ratio in patient specific cells. To investigate the molecular mechanisms responsible for the fate choices neural stem cells take during early differentiation we have utilised single cells RNA sequencing. Using various bioinformatics methods we can timely model the gene expression in neural cells when they enter or leave a fate choice decision. We have found that cell adhesion molecules are very important for the various fate choices. We hope that this on-going study will reveal genes and signalling pathways important to make the proper choices of fate by neural stem cells and how diseased cells fail, which might explain the cause of some neurodevelopmental disorders.

T-1118

EPITRANSCRIPTOMIC CONTROL OF EMBRYONIC CORTICAL NEUROGENESIS

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The N6-methyl-adenosine (m6A) modification has recently garnered interest due to its regulatory functions in embryonic stem cell fate transitions in vitro. This dynamic methylation occurs abundantly on mRNAs, including on transcripts for transcription factors involved in differentiation and pluripotency. The m6A tag has been shown to affect RNA stability, translation, translocation and splicing, however the in vivo function of m6A methylation in mammalian systems remain unknown. Here we investigate the role of m6A on neural stem cell biology in vivo using conditional knockout (cKO) mice of Mettl14, a subunit of the m6A methylation complex. Loss of Mettl14 function in the developing cerebral cortex results in marked alteration of the proliferative and self-renewal capabilities of neural stem cells and neural progenitors. Notably, m6A depletion results in the persistence of neurogenic zones with radial glial cells in the postnatal cortex. Live imaging experiments show a 1.5-fold increase in the length of the cell cycle of Mettl14 cKO cultured neural progenitors relative to the wildtype. Both in-vivo and in-vitro analysis show a significant delay in the progression from S to M phase in the absence of m6A methylation. Genome-wide m6A sequencing analysis revealed enrichment of the m6A

modification in neural stem cell-specific genes and cell cycle regulatory genes. In parallel, RNA-seq analysis showed an increased abundance of the transcripts normally modified with m6A in the knockout animals compared to the wildtype. This supported the idea that m6A modulates RNA stability, which we confirmed using a stability assay in neural progenitor cells. Together, our analysis reveals a novel role of m6A methylation in regulating neural stem cells and neurogenesis in the mammalian brain in vivo.

T-1120

PRECONDITIONING OF HUMAN IPSC-DERIVED NEURAL STEM CELLS WITH METFORMIN TO PROMOTE POST-STROKE RECOVERY

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The generation of human induced pluripotent stem cells (hiPSCs) from human fibroblasts has revolutionized cell therapy by providing a source of autologous cells for transplantation. Several studies have demonstrated that transplantation of hiPSC-derived neural stem cells (hiPSC-NSCs) increases regeneration and recovery following stroke, supporting their therapeutic potential. However, major concerns for translating hiPSC transplantation therapy to the clinic are efficacy and safety. Therefore, there is demand to develop an optimal strategy to enhance the engraftment and regenerative capacity of transplanted hiPSC-NSCs. The recent published work shows that metformin, an FDA approved drug, is an optimal neuroregenerative agent by promoting proliferation and differentiation of neural stem cells and limiting tumorigenesis following transplantation. In this regard, we hypothesize that preconditioning of hiPSC-NSCs with metformin before transplantation into the stroke-damaged brain will improve engraftment and regenerative capabilities of hiPSC-NSCs, ultimately enhancing functional recovery. Here we show treatment of hiPSC-NSCs in vitro with metformin enhances the proliferation and differentiation of hiPSC-NSCs even after withdrawal of metformin treatment, showing its promise as a novel preconditioning strategy. Future experiments will determine whether preconditioning hiPSC-NSCs with metformin before transplantation will enhance their survival and regenerative capability following transplantation into a rat stroke model. Preconditioned and naïve hiPSC-NSCs show no difference in engraftment 1 week after transplantation into an endothelin-1 focal model of ischemia. After 8 weeks post-transplantation, we will assess proliferation and differentiation of hiPSC-NSCs in addition to functional recovery through behavioural tasks. These studies represent a vital step in the optimization of hiPSC-based replacement therapy to enhance post-stroke recovery.

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T-1122

DIFFERENTIAL MTORC1 ACTIVITY IN SUBDOMAINS OF THE MOUSE VENTRICULAR-SUBVENTRICULAR ZONE LEADS TO LOCATION SPECIFIC TUMOR DEVELOPMENT

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The ventricular-subventricular zone (V-SVZ) is the largest neurogenic niche in the postnatal brain. Recent studies have revealed extensive heterogeneity even within cell types in this region previously thought to be monolithic. We now know that V-SVZ neural stem/progenitor cells (NSPCs) have a positional identity: their original location within the stem cell niche defines the type of progeny produced. Positional identity can thus be discerned by measuring cell location within the niche or expression of region-specific transcription factors. However, it is less clear if signaling responses within subpopulations of NSPCs define their local proliferation and behavior. If signaling indeed controls identity, it may be possible to productively modulate signaling in these cells to change their identity. To test this idea, we developed intracellular phospho-specific flow cytometry (phospho-flow) to measure signaling in individual cells and determine whether signaling controls stem cell behavior in normal brain and disease models. The focus of this study was the mTOR signaling pathway, which is disrupted in multiple neurodevelopmental disorders and brain tumors. NSPCs within the ventral V-SVZ displayed elevated basal mTORc1 pathway activity relative to dorsal counterparts. As mTORc1 activity is coupled with increased cell size and protein translation, this suggested that specific NSPC subpopulations may form localized brain tumors in the mTOR-associated disorder Tuberous Sclerosis Complex (TSC). To test this, embryonic region-specific drivers were used to remove Tsc2, an upstream mTORc1 inhibitor, in dorsal or ventral V-SVZ. Loss of Tsc2 in the ventral V-SVZ resulted in tumor formation within the V-SVZ stem cell niche, while removal of Tsc2 in the dorsal V-SVZ had no tumor phenotype. These findings support the hypothesis that location of origin signaling underlies tumor development in TSC.

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T-1124

IN VIVO INHIBITION OF BMP AND SFRP2 IN THE ADULT MOUSE EYE INDUCES RETINAL STEM CELL PROLIFERATION, EXPANSION AND ENHANCED GROWTH FACTOR STIMULATION

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Adult retinal stem cells (RSCs) are rare cells that reside in the pigmented ciliary epithelium (CE) of the mammalian eye. In culture, RSCs have the capacity to self-renew and differentiate into all of the cell types of the neural retina and retinal pigmented epithelium, and readily proliferate to form clonal, free-floating spheres in 7 days. However, in vivo, RSCs do not proliferate or generate new retinal cells in adult mammals. Previously, we identified BMP and sFRP2 proteins as mediators of adult RSC quiescence with in vitro experiments. Here, we investigated whether BMP and sFRP2 inhibition could induce RSC proliferation in vivo in mice. Intravitreal injections of the BMP antagonist Noggin or an anti-sFRP2 antibody were administered once a day for 3 days, with EdU present in the drinking water. 1 day post-injection (DPI), each factor induced a ciliary body (CB)-specific increase in EdU labeled cells. At 1 DPI, CE cells, identified by Pax6 labeling, showed a ~5-fold increase in EdU+ cells per µm2 for each factor. Combined Noggin and anti-sFRP2 injection resulted in a 6.5-fold increase in EdU+/Pax6+ CE cells per µm2 versus control but did not have an additive or synergistic effect, suggesting both factors may converge on the same pathway to induce CE proliferation. In contrast, Noggin and anti-sFRP2 combined with growth factors (GFs) FGF and insulin did induce an additive increase in EdU+/Pax6+ cells per µm2 (~12 fold). Surprisingly, GFs alone showed a ~5-fold increase in EdU+/Pax6+ cells. At 31 DPI, the EdU+/Pax6+ population persisted and no Ki67 staining was observed, indicating these cells survive, but do not continuously divide. At both 7 and 31 days after Noggin or anti-sFRP2 injection, clonal sphere assays revealed an over two-fold increase in the number of sphere-forming RSCs present in the eye. Together, these results demonstrate that blocking BMP and sFRP2 in the adult mouse eye can bring RSCs out of quiescence, induce them to proliferate and expand, and enhance their response to growth factors. However, it remains to be determined if this activated RSC population can differentiate into new retinal neurons, migrate into the neural retina and improve visual function.

Funding Source: Canadian Institutes of Health Research, Medicine by Design, Foundation Fighting Blindness

T-1126

SOX2 AND LIN28 PROMOTE PROLIFERATION AND CELL FATE OF INNER EAR NEUROSPHERES IN VITRO

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Degeneration of the auditory nerve is an important cause of sensorineural hearing loss that can be caused by auditory neuropathy or genetically inherited diseases. As auditory neurons do not regenerate spontaneously, our regeneration therapies focus on restoration of hearing by regenerating auditory neurons from glial cells that remain after the loss of auditory neurons. Sox2, a bHLH transcription factor, interacts with mRNA-binding protein Lin28 in neural stem cells to maintain optimal levels of Lin28 and promote proliferation of progenitor cells. In the postnatal inner ear Sox2 has previously been associated with expansion of sensory progenitors that develop into hair cells, the sensory epithelium of the inner ear. We found that proteolipid protein 1 (Plp1), a marker for glial cells that can act as progenitor cells for neurons in the CNS, is coexpressed with Sox2 in peripheral glia of the inner ear. Furthermore, Plp1-expressing neurospheres were dependent on Sox2 and Lin28 for renewal and proliferation. Plp1- positive cells were isolated from neonatal inner ears of Sox2- or Lin28 deficient mice (Plp-Cre-ER;Sox2flox/flox, Plp-Cre-ER;Lin28aflox/flox), or from Sox2- or Lin28-overexpressing mice (tetO-Sox2;PLP-tTA, or tetO-Lin28;PLP-tTA). Floating neurospheres were generated in vitro, and quantified after 3 passages. Differentiating neurospheres were analyzed for neural progenitor and glial cell marker expression by quantitative RT-PCR and immunohistochemistry after 9 days in vitro. Lineage tracing for Sox2 or Lin28 revealed reduced proliferation and failed neural induction after downregulation in Plp1-positive neurospheres. Transient Doxycycline controlled overexpression of Sox2 or Lin28 was followed by increased proliferation and neurogenesis from Plp-positive progenitors in vitro. We hypothesize that glial cells of the inner ear function as stem cell pool for inner ear neurons. This study is a powerful experimental approach that allows us to answer questions about the key signals for neurogenesis and regeneration of auditory neurons, which may ultimately lead to novel, biological treatments for hearing loss.

NEURAL DISEASE AND DEGENERATION

T-1132

ADULT HUMAN BONE MARROW MESENCHYMAL STEM CELLS ATTENUATE SPINAL CORD ISCHEMIA-REPERFUSION INJURY IN A MOUSE MODEL

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Paraplegia induced by spinal cord ischemia reperfusion injury (SCIR) remains a devastating complication following thoracic aortic surgeries/interventions and no effective treatment has never been established. Recently, therapeutic efficacy of human bone marrow mesenchymal stem cell (hBM-MSC) has been preclinically demonstrated in traumatic spinal cord injury, however its efficacy for SCIR is unknown. Female C57BL/6 mice were subjected to SCIR by occlusion of the aortic arch and left subclavian artery for 5.5 minutes. Two hours after SCIR, murine were randomly allocated to receive intravenous injection of 5x10^6/kg hBM-MSCs (cell group, N=9) or phosphate-buffered saline (control group, N=7). No immunosuppressant was administrated after the cell injection. Motor function was assessed using the Basso Mouse Scale (BMS) scoring after SCIR. Immunohistochemical staining for NeuN was performed to detect motor neurons in the spinal cord at week 4. Mortality within 4 weeks after SCIR was similar in both treatment groups. BMS score 0 and 2 hours after SCIR was 0 indicating complete paraplegia in all mice in both groups. In the survived animals, BMS score was 0 throughout the study period in the control group, whereas BMS score was 9 indicating normal function in all mice in the cell group at week 1 or later. Change in BMS score between hour 2 and hour 24 was significantly greater in the cell group than the control group (median, 8.0; range 4.0-9.0 vs 0.0; 0.0-0.0, P=0.0004). Changes in BMS score between hour 2 and week 1 was also greater in the cell group than the control group (9.0; 9.0-9.0 vs 0.0; 0.0-0.0, P=0.03). Average number of motor neurons in the 3 histological sections of lumber spinal cord at week 4 tended to be greater in the cell group than the control group (23.6±3.3 vs 5.6±3.1; P=0.08). In addition, to confirm the localization of the transplanted cells, CM-DiI-labeled cells (N=2) were intravenously injected at hour 2 after SCIR. DiI-labeled hBM-MSCs were abundantly localized in the ventral horn of the lumber spinal cord at hour 8. In conclusion, intravenous administration of hBM-MSCs may potentially attenuate SCIR by preserving motor neurons at the site of cell incorporation in the spinal cord.

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T-1134

QUANTITATIVE LIVE-CELL ANALYSIS FOR OPTIMIZATION OF CULTURE CONDITIONS AND EVALUATION OF CELL HEALTH IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONS

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A major limitation in studying human diseases affecting the nervous system is the ability to culture, monitor and analyze neuronal cells that accurately represent human phenotypes of these disorders. The use of human induced pluripotent stem cell (hiPSC)-derived neurons has provided a new approach aimed at modeling neurological diseases. Monitoring neuronal morphology and cell health in long-term culture is critical for the characterization and evaluation of these novel model systems. Traditional approaches rely on endpoint assays and imaging techniques that require immunochemical staining, which provide limited real-time kinetic information. Here we present data highlighting optimal culture conditions for cell viability and neurite outgrowth in hiPSC-derived neurons from Cellular Dynamic International (CDI, iCell Neurons). We also evaluated neurite outgrowth and cellular viability in iCell Gluta Neurons from CDI using a quantitative, live-cell imaging approach with the IncuCyte ® Live-Cell Analysis System over days/weeks in culture. Testing multiple culture and plate coating conditions, we find iCell Neurons respond best with a combination of BrainPhys media (Stem Cell Technologies) and iCell DOPA Neuron Supplement/ iCell Nervous System Supplement (CDI) combined with a plate coating of polyethyleneimine and laminin. To exemplify a real-time imaging approach using hiPSC-derived neurons, we evaluated glutamate- and kainate-induced excitotoxicity using the IncuCyte ® phase/ fluorescent NeuroTrack applications multiplexed with Annexin V reagents in iCell Gluta Neurons. Glutamate and kainate produced a concentration- and time-dependent decrease in neurite length with a concomitant increase in red or green object count (indicating cell death) over 72 hours. Glutamate and kainate produced a similar effect when measured by IncuCyte™ Cytotox Red, IncuCyte™ Cytotox Green and caspase 3/7 reagents. Treatment with the NMDA receptor antagonist MK-801 and the AMPA receptor antagonist NBQX reduced the glutamate- and kainate-induced effects on neurite length and cell death.

These data outline optimal culture conditions for iCell Neurons and demonstrate the ability of the IncuCyte ® approach for real-time, long-term quantitative analysis of iPSC-derived neuronal cell health.

T-1136

GENOTYPE/PHENOTYPE CORRELATION IN HUMAN IPSC-DERIVED NEURONAL NETWORKS - IN VITRO HTS DISEASE MODELING WITH MEA'S COUPLED WITH OMICS ANALYSES

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To decrease attrition rates is one of the major challenges of drug discovery. One strategy is the development of more predictive pre-clinical in vitro models. Human induced pluripotent stem cell-derived (hiPSC) neuronal cultures promise higher physiological relevance and thus, better translation to the in vivo situation. In this context genetic cell models have been designed or produced from patient biopsies. However, one of the biggest concerns is their physiological relevance needed for disease modeling. Our aims were a) to characterize the correlation between known disease genotypes (e.g. of familiar Alzheimer's disease) and their functional in vitro phenotype and b) to better understand these human iPSC neuronal in vitro models by comparing them with primary mouse neuronal cell cultures. We used commercially-available human iPSC-derived neuronal networks partly containing Alzheimer's disease-relevant risk gene APP mutations and cultured them on HTS-compatible multiwell micro-electrode arrays (MEA) for multiple weeks to analyze their functional electrophysiological activity patterns quantified by multi-parametric analysis. After recording, the cells were prepared for proteome and transcriptome analyses. By comparing human functional phenotypes with those from different specific mouse brain regions we show that human neuronal cell exhibit specific and reproducible phenotypic similarity profiles (e.g. midbrain or hippocampus-like). Genetic or toxin-induced manipulation affects these activity patterns thereby producing specific and significant functional phenotypes (alpha-synuclein treatment, mutation in APP gene). For APP-mutated cells our OMICs data showed regulation of more than 100 genes including genes involved in cell cycle, glutamate receptor signaling or neuroprotection. In conclusion, we show that hiPSC neuronal are able to produce meaningful functional in vitro phenotypes, that these phenotypes can be changed by disease-associated modulation and we provide first data for a genotype/ transcriptome/ phenotype correlation.

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T-1138

PROBING DEVELOPMENTAL CONSEQUENCES OF PSEN1 MUTATIONS IN IPSC DIFFERENTIATION IN 2D AND 3D

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Mutations in the gamma secretase subunits Presenilin 1/2 (PSEN1 and PSEN2) lead to familial Alzheimer's disease (fAD). In addition to cleavage of amyloid precursor protein (APP), gamma secretase cleaves Notch and other transmembrane proteins. Notch signalling has a crucial role in normal development, stem cell differentiation and cellular migration. Using patient-derived iPSCs, we are investigating the effects of fAD PSEN1 mutations on stem cell differentiation and early pathological processes. We have generated a panel of fAD iPSC lines via episomal reprogramming (Okita et. al. 2011) from patients with the PSEN1 mutations Δintron 4, M139V, R278I, Y115H, P264L and E280G. Together with iPSCs from healthy controls, these lines were subjected to a cortical differentiation protocol (Shi et. al. 2012) and a cortical organoid differentiation protocol (Lancaster et. al. 2013) to generate glutamatergic neurons in 2 and 3 dimensions. iPSC lines were subjected to a number of quality control checks including expression of pluripotency markers, karyotypic stability and absence of reprogramming episome integration. Successful cortical differentiation was confirmed via presence of multiple cortical layer markers with similar efficiency shown between lines. Development timings appear affected in PSEN1 mutant lines and we are investigating the role of Notch signalling in neuronal terminal differentiation. Work is ongoing into the early pathological processes in these cells such as cellular migration as well as exit from the cell cycle. Our panel of control and patient-derived PSEN1-mutant iPSC lines enable investigations into the effects of fAD mutations on Notch signalling and subsequent differentiation properties in our cell lines. These will have implications for research into the developmental effects of fAD mutations and could broaden the scope for future drug targets and clinical strategies in Alzheimer's disease.

T-1140

IDENTIFYING DIFFERENTIALLY EXPRESSED TRANSCRIPTS BETWEEN SNC AND VTA DOPAMINERGIC NEURONS IN THE BABOON: A NON-HUMAN PRIMATE MODEL

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The progressive death of dopamine producing neurons in the substantia nigra pars compacta (SNc), but not in the closely related ventral tegmental area (VTA), is the principle cause of Parkinson's disease (PD). Dopaminergic (DA) neurons produced from induced pluripotent stem cells provide a promising avenue for a patient-specific, stem cell-based replacement therapy. Current transplant protocols do not distinguish the type of DA cell transplanted but post mortem analysis suggest that SNc survive preferentially. Therefore, transplant success may be improved by transplant of purified SNc type neurons. These studies are hindered by the lack of well-defined markers distinguishing DA neurons of these two types. Data from meta-analyses of differentially regulated transcripts from the equivalent regions of rodent brains as well as single cell analysis demonstrates differences between this species and humans. Nonhuman primates provide the most accurate preclinical models in which to optimize the efficacy and safety of stem cell-based transplantation therapies for neurodegenerative disease. We differentiated baboon fibroblast-derived iPSCs (biPSCs) into dopaminergic neurons and confirmed their midbrain dopamine neuron phenotype on the basis of cell-type specific expression of dopamine markers, RT-qPCR, perforated patch-clamp electrophysiology, and fast scan cyclic voltammetry. However, this data does not allow us to distinguish SNc-like neurons from VTA-like neurons. To address this question, we first compared population-level expression of the baboon midbrain and compared it with the human data set. We show that 9 of 10 highly differentially regulated transcripts in human midbrains were also differentially regulated in baboons. Single-cell transcriptomics yields high resolution data, from which specific cellular and molecular phenotypes of subpopulations of midbrain dopaminergic neurons may be identified. Such information will be required to facilitate the selective derivation of SNc-like neurons. Our preliminary studies support our contention that the baboon can provide a valuable preclinical model for testing and optimizing the efficacy and safety of stem cell-based therapeutic approaches for the treatment of PD.

T-1142

AN INTERACTION NETWORK OF RISK FACTORS INVOLVED IN PSYCHIATRIC AND NEURODEVELOPMENTAL DISEASES

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Genetic studies of psychiatric disorders have provided us with putative risk factors implicated in those disorders. A 2014 GWAS study of schizophrenia has implicated 108 gene loci in the disease with some of the loci containing only a single gene and some containing multiple genes, giving estimates of more than 400 putative risk genes. Given this plethora of putative risk factors, we decided to build an interaction network of the risk proteins with the aims of (1) identifying molecular interactors of each of the risk factors, (2) uncover risk factors that are connected through their interactors and thus potentially are part of a pathological pathway, and (3) discover risk factors from multi-genic loci that are part of the network and thus potentially the relevant gene for the disease in that locus. To build the network we took the approach of performing co-immunoprecipitaton (co-IP) of risk proteins from human induced Ngn2 cortical glutamtergic excitatory-like (iNgn2) neurons. We started our study with the schizophrenia GWAS hits, prioritizing risk proteins based on whether they were in a single gene locus, have been implicated in psychiatric disorders in other studies, and were expressed in iNgn2 neurons. To start, we performed co-IPs for CACNA1C and HCN1 using antibodies against endogenous proteins. We co-IPed HCN1 in 50 day iNgn2 neurons and discovered 524 proteins which were enriched in the HCN1 IP versus control IgG IP with an FDR < 0.1. This is considerably more than just the 7 interactors found in InWeb (a scored human protein-protein interaction network of existing interactions found in literature) [ref]. Among the 524 interactors, we identified 26 proteins previously implicated in schizophrenia, autism or developmental disorders. For CACNA1C we performed co-IPs over a timecourse of neuronal differentiation at days 19, 22, 29, and 50. We identified a total of 304 interactors (FDR < 0.1) enriched in any of the time-points as compared to 54 of the known interactors of CACNA1C found in InWeb. Furthermore, among the 304 interactors we identified 21 interactors implicated in other psychiatric or developmental disorders. These data show that performing IPs in human iNgn2 neurons uncovers relevant novel interactions of risk proteins and we are

expanding this approach to other risk factors involved in schizophrenia.

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T-1144

ENHANCEMENT OF NEURAXIS COVERAGE BY TRANSPLANTED MULTIPOTENT HUMAN NEURAL STEM CELLS IN AN ALS MOUSE MODEL USING FUCOSYLATION AND/OR A CXCR4 AGONIST

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Amyotrophic Lateral Sclerosis (ALS) is a degenerative motor neuron disease that leads to early death with no currently effective treatment options. We have reported that the multiple actions of neural stem cells, including those of human origin (hNSCs), transplanted into a mouse model of ALS have been able to address the multiple pathologies associated with ALS, forestalling disease-onset and significantly prolonging symptom-free survival. Their degree of effectiveness is dependent upon the extent of neuraxis coverage and is limited by the invasiveness of therapeutic procedures. For every 1 unit increase in the number of regions of the spinal cord covered there is a 30% decrease in risk-of-death ratio. To enhance this neuraxis coverage, we propose three minimally invasive procedures for improving outcomes in the SOD1G93A mouse model. The first is to fucosylate hNSCs using alpha- (1,3)- fucosyltransferase (FT-VI) to allow intravascular administration of the hNSCs and enhance transvascular passage, homing, and engraftment of the cells throughout the spinal ventral horns. The second is to use a synthetic peptide agonist of the chemokine receptor CXCR4 (a detoxified version of SDF-1α called "SDF-V1") to direct the pathotropic homing of intraspinally transplanted hNSCs to widely distributed regions of need. The third is to use a combination of the two approaches, assessing their degree of synergy. Using histological and immunohistochemical assessment, in vivo bioluminescent imaging, behavioral/motor assessments, and electrophysiological studies, we will determine the extent to which fucosylated hNSCs enhance vascular transmigration of infused hNSCs above the 5% observed for untreated hNSCs; an optimal dose of FT-VI will be determined. We are also in the process of determining the degree to which SDF-VI increases the coverage area of hNSCs stereotactically implanted either into the central canal or into the ventral horns in a small number of selected segments of the early symptomatic SOD1G93A mouse spinal cord. Given that clinical trials using hNSC implantation in patients with ALS are presently ongoing, efficacy and safety of these elaborations in mice, may suggest adding these approaches in order to enhance and optimize transplantation based treatments in patients afflicted with this debilitating disease.

T-1146

ESTABLISHING A CRISPR-BASED PLATFORM TO STUDY SCHIZOPHRENIA-ASSOCIATED GENES IN HUMAN NEURONS

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Schizophrenia (SZ) is a complex genetic neuropsychiatric disease inherited via both common and rare polygenic risk factors. SZ genome wide association studies (GWAS) have identified many SZ-associated single nucleotide polymorphisms (SNPs) positioned in the putative enhancer regions of neuronal genes, suggesting a link between these SNPs, their respective neighboring gene(s), and SZ risk. Recently, the CommonMind Consortium (CMC) examined gene expression in post-mortem brains, identifying five genes with the strongest correlation between genotype and brain expression levels: FURIN, SNAP91, CLCN3, TSNARE1 and CNTN4 (herein referred to as the "CMC genes"); however, the functional role of these five genes in post-mitotic human neurons remains unresolved. We recently adapted a scalable CRISPR activation and interference (CRISPRa and CRISPRi, respectively) platform to NGN2-induced excitatory neurons, enabling robust and combinatorial manipulation of CMC gene expression in human excitatory neurons. Specifically, we generated CRISPRa (dCas9-VPR) and CRISPRi (dCas9-KRAB) stable NPC lines to achieve consistent expression of dCas9-effectors, and then validated lentiviral-expressed gRNAs for each gene in NGN2-neurons generated from 3 control individuals. RNAseq-based comparison of excitatory neurons with altered expression of CMC genes is underway, facilitating a greater understanding of how perturbed expression of these CMC genes may contribute to SZ risk. In parallel, multi-electrode array and calcium imaging experiments are evaluating the functional effect of manipulating these CMC genes in excitatory neurons. Our goal is to better understand the link between these CMC genes, neuronal activity and SZ risk.

T-1148

INVESTIGATING STEM CELL ACTIVATION STRATEGIES TO IMPROVE COGNITIVE DEFICITS FOLLOWING MPFC STROKE

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Cognitive impairments are prevalent following clinical stroke; however, preclinical research has focused almost exclusively on motor deficits. We recently developed a focal model of stroke affecting the medial prefrontal cortex (mPFC) that results in deficits in executive function and memory processing. Two commonly used drugs, cyclosporin A (CsA) and metformin (Met) have been shown to improve motor deficits following stroke, and are associated with the activation of endogenous neural precursor cells (NPCs) and increased survival and migration of newly born cells. Here, we investigated the potential of these activation strategies to enhance cognitive recovery following injury. Adult male C57Bl/6 mice were subjected to surgical stroke via bilateral injection of endothelin-1 into the mPFC (or a sham procedure). Beginning 24 hours later, CsA, Met, or vehicle was delivered via subcutaneous pump for 3-8 weeks. Following drug treatment, animals were subjected to a battery of cognitive tests designed to assess problem solving and memory function. These included object recognition, open field, puzzle box task, Morris Water Maze (MWM), and spontaneous alternation. Subsets of animals were sacrificed at early and late time points and the mPFC and lateral ventricles were dissected and assayed for the presence of neural stem cells using the in vitro neurosphere assay. Additional animals were perfused and tissue was collected for infarct volume and immunohistochemical analyses. mPFC stroke resulted in persistent behavioural deficits apparent several weeks following injury, and significantly expanded the NPC pool 1 week after stroke. CsA treatment did not further enhance the NPC pool compared to stroke alone or improve cognitive recovery; however, there was evidence of increased migration of NPCs toward the injury following CsA treatment (NPCs evident in the injured cortex of 43% vs 14% of mice). Met treatment appears to improve long-term memory deficits and problem-solving; analyses are ongoing to characterize the corresponding neurogenic effects. These data suggest that activation of NPCs may have potential to improve recovery of cognitive function, an important and understudied area of stroke research.

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T-1150

FUNCTIONAL INVESTIGATION OF RARE GENETIC VARIANTS ASSOCIATED WITH A-SYNUCLEIN PATHOLOGY AND PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disorder affecting 6 million people worldwide. A cellular hallmark of PD is the presence of abnormal aggregates of the protein -synuclein (-syn). A substantial component of PD is thought to be heritable, but most of this heritability is currently unexplained. Tremendous advances in genome sequencing promise to uncover variants that account for this "missing heritability", but the question of how to distinguish pathogenic and non-pathogenic variants has emerged as a pressing one for the field. Rare variants require exceedingly large sample sizes to achieve enough statistical power, and recent human genetic divergence limits the use of replication across populations as a way to validate them. We previously used unbiased screens to uncover a network of genes that impact -syn cellular toxicity. These studies identified known PD genes, suggesting that additional genes in our network may also be risk factors. We therefore executed targeted exome sequencing of these genes in ~500 patients with -synucleinopathies including PD. We describe here a cross-species platform to stratify variants according to relevant biological activities. First, this platform interrogates the ability of these variants to modulate -syn toxicity in two complementary cellular systems—the simple and genetically tractable baker's yeast, and more complex human neurons derived from PD patients in which candidate variants are introduced through CRISPR/Cas9-dependent editing. Second, we interrogate the effect of variants on molecular interactions at proteome scale ("edgotype"). This multi-faceted platform promises to shed new light on genetic risk factors for PD. More generally, we present a rational biological approach for distinguishing normal from pathogenic gene variants and the stratification of patients according to molecular mechanism.

T-1152

IDENTIFICATION OF ALS DISEASE GENE EXPRESSION AND ELECTROPHYSIOLOGICAL SIGNATURES IN SINGLE MOTOR NEURONS USING PATCH-SEQ

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Amyotrophic lateral sclerosis is a devastating, progressive neurodegenerative disease that results in the loss of both the upper and the lower motor neurons (MN). The disease manifests itself in both familiar and sporadic forms with the latter accounting for around 90% of ALS cases. A number of genes have been associated with ALS such as SOD1, TDP43, FUS or C9ORF72 with hundreds of different mutations detected within this pool of genes. These genes are important to mitochondrial function, are RNA-binding proteins or have unknown functions to date. Furthermore, clinical neurophysiological studies revealed that ALS patients exhibit a hyperexcitability phenotype in their neuromotor circuit. Importantly, so far no unifying hypothesis has been developed that explains how mutations of these genes with different functions converge into the ALS disease progression observed in patients. In order to elucidate the correlation between these different molecular and functional hallmarks of ALS, we decided to use patch-seq analysis to study both the gene expression and the electrophysiological profile of single, ALS-affected motor neurons. To execute this experimental approach, we corrected the ALS associated A4V mutation in superoxide dismutase 1 (SOD1A4V/+) in patient-specific iPS cells and from these cell lines generated motor neuron reporter cells to allow for the specific isolation of single motor neurons. After isolation and a three-week maturation period in the presence of glia cells we assessed and compared single human motor neurons carrying either the SOD1A4V/+ or the corrected SOD1+/+ gene using patch clamp. Immediately after acquisition of their electrophysiological properties the motor neurons were collected and their gene expression analyzed using single-cell RNAseq. Similar to the hyperexcitability phenotype detected in ALS patients, we observed that SOD1A4V/+ motor neurons were hyperexcitable compared to isogenic, SOD1+/+ MNs. Interestingly, the patch-seq analyses reveal different, unique genetic signatures shared by as well as unique gene signatures that distinguish between isogenic MNs which seem to drive the differential electrophysiological properties. We hope that our ongoing analysis of this study will provide novel insights into the cause and progression of ALS disease.

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T-1154

THE EXPRESSION OF ALZHEIMER'S DISEASE ASSOCIATED GENES IN IPSCS DERIVED FROM PERIPHERAL BLOOD MONONUCLEAR CELLS

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Alzheimer's disease(AD) is one of the most common neurodegenerative diseases. It was reported that mutations in amyloidprecursor protein(APP), presenilin1(PSEN1) and presenilin2(PSEN2) caused most of the early-onset AD, while the ε4 allele of the apolipoprotein E (APOE) gene is the strongest risk factor for late-onset AD. However, little is known about the gene expression of these genes in AD patients. To determine whether the expression of these genes was altered in AD patients, we conducted this study using induced pluripotent stem cells (iPSCs) derived from AD patients. Human peripheral blood mononuclear cells (PBMC) from 17 AD patients and 8 healthy subjects were reprogrammed using non-foot print technology. Footprint-free iPSCs were stained positive for alkaline phosphatase and the expression of pluripotency markers (OCT4, SSEA4, TRA-1-60, NANOG) was visualized by immunofluorescent staining. Western blot was performed to verify protein expression of OCT4 and NANOG in iPSCs. Total RNA from iPSCs was isolated using Trizol reagent and 1 g of total RNA was reverse transcribed into cDNA using SuperScript VILO Master Mix. Real-time quantitative PCR (Q-PCR) analysis was performed using 7500-Fast Real-Time PCR Systems with gene specific primers and SYBR Green Master Mix (Life Technologies). Gene expression levels were normalized by -actin. More than 90% cells expressed the stem cell marker Oct4, Nanog, Sox2 and SSEA4. Karyotype analysis confirmed that iPSCs carry a normal karyotype during the process of generations. There was no significant difference detected in the mRNA expression levels of APP (1.290±0.039 in control vs 1.259±0.078 in AD patients), PSEN1 (1.027±0.028 in control vs 0.970±0.036 in AD patients), PSEN2 (1.408±0.099 in control vs 1.088±0.102 in AD patients) and APOE (1.207±0.028 in control vs 1.109±0.053in AD patients), between iPSCs derived from AD patients and normal control groups. In conclusion, the was no difference in the basic mRNA expression levels of AD

associated genes APP, PSEN1, PSEN2 and APOE in iPSCs derived from AD patients and normal control subjects. We will conduct further studies such as 3D culture or differentiation of these iPSCs into neuron cells to test whether these is altered expression of these genes in AD patient-derived iPSCs.

CANCERS

T-1158

H3K9 METHYLTRANSFERASES AND DEMETHYLASES CONTROL LUNG TUMOR PROPAGATING CELLS AND LUNG STEM CELLS

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A significant gap in our understanding of stem cells in cancer is the extent to which their regulatory mechanisms are conserved or dysregulated compared to stem cells of the same healthy tissue. We have sought to directly address this by searching for potential common regulators of murine lung stem cells and cancer stem cell-like tumor propagating cells (TPCs). We have identified the H3K9me1/2 methyltransferases G9a/Glp as negative regulators of TPCs by screening lung adenocarcinoma cells for small molecules that modulate the TPC surface antigens CD24 and Sca-1. Gene expression analyses of primary adenocarcinomas confirm that G9a/Glp are down-regulated in Sca-1+ TPCs. Chemically inhibiting G9a/Glp promotes TPC phenotypes in lung adenocarcinoma cells, increasing their in vitro organoid forming efficiency and widening the distribution of tumors formed by inhibited cells after intravenous transplantation. Depleting G9a during tumorigenesis significantly alters tumor population dynamics, enriching for TPCs and consequently accelerating disease progression and metastasis. Furthermore, inhibition of G9a/Glp in 3D multipotent lung stem cell cultures disrupts the normal formation of differentiated organoids, specifically impairing alveolar lineage organoids. This suggests a potential parallel role for H3K9me1/2 in controlling the dynamic between stem cells and certain differentiated cells in both the healthy lung and in lung cancer. Analysis of 400+ early stage patient lung adenocarcinomas reveals that low G9a expression and high expression of KDM3A, an H3K9me1/2 demethylase (KDM), significantly correlate with worse survival (P=0.008, P=0.002), implying that dysregulation of H3K9me1/2 is also a significant factor in human disease. Preliminary data suggest that depleting H3K9 KDMs downregulates TPC markers in lung adenocarcinoma cells and decreases their organoid forming efficiency, raising the possibility that targeting these molecules in lung adenocarcinoma patients may be therapeutically beneficial.

T-1160

MODELING GATA2 DEFICIENCY ASSOCIATED HEMATOLOGIC MALIGNANCIES IN ZEBRAFISH

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Myeloid neosplasms, such as myelodysplastic syndromes (MDS) and acute myeloid leukemia AML), arise from abnormal hematopoietic stem and progenitor cells (HSPCs) with acquired somatic mutations. A considerable portion of these hematologic malignancies are the result of cancer susceptibility syndromes due to germline mutations. GATA2 haploinsufficiency is one such cancer predisposition state associated with an autosomal dominant familial MDS/AML syndrome and a high prevalence of myeloid neoplasms. We modeled gata2 deficiency in zebrafish to study the preleukemic hematopoiesis, particularly focusing on the consequences of cooperating mutations. To induce gata2 deficiency associated hematologic malignancies, we introduced combinations of genetic mutations described in MDS and AML. Loss of function mutations were mosaically introduced with CRISPR/Cas9 technology, while overexpression of dominant negative IDH2R140Q was achieved via transgenesis under the zebrafish blood-specific promoter draculin. Zebrafish embryos were injected with guide RNAs targeting zebrafish orthologs of gata2 and various combinations of additional mutations in tet2, asxl1 and IDH2R140Q, and grown to adulthood. Kidney marrow cells of adult zebrafish were analyzed at various time points between 3-8 months post-fertilization (mpf) by flow cytometry and cytospins. Mosaic combinations of mutations in the presence of gata2 deficiency resulted in the expansion of progenitor population in the kidney marrow with blast-like cells at 8 mpf. A concomittant decrease in the myeloid population was noted, without any changes in the lymphoid population. The majority of zebrafish with leukemoid transformation harbored mutations in asxl1 in addition to gata2. We have successfully modeled early stages of gata2 deficiency associated hematologic malignancies in the presence of asxl1 deletion in zebrafish.

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T-1162

THE FIMBRIAL END OF MURINE FALLOPIAN TUBE IS ENRICHED WITH STEM AND PROGENITOR CELLS FOR THE FALLOPIAN TUBE EPITHELIUM

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Epithelial ovarian cancer (EOC) remains the most lethal malignancy of the female reproductive system, mainly due to the fact that most EOCs are diagnosed at advanced stages. Thus, a better understanding of the cellular origin and early stages of EOC should have important implications for the improved diagnosis, treatment and prevention of this malignancy. Recent studies suggested that most EOCs might originate from transformation of fallopian tube epithelial (FTE) cells. The fallopian tube (FT) epithelium is mainly composed of ciliated and secretory cells. However, whether and how these two types of FTE cells are sustained by FTE stem/progenitor cells, what is the relation of the putative FTE stem/progenitor cells to the cellular origin of EOC, and how EOC originates from FTE cells remain largely elusive. To address these, we performed lineage-tracing studies to map the in vivo fate of FTE cells using two approaches: one was based on intrabursal injection of Cre-expressing adenovirus under the control of the Keratin 8 promoter (Ad-K8-Cre), which mainly targets FTE cells in the distal (fimbrial) portion of the FT; the other was based on the K8-CreER mouse line (upon induction by tamoxifen), which targets FTE cells in both the distal (i.e., closer to the ovary) and proximal (i.e., closer to the uterus) portions of the FT. We found that while both approaches provided evidence for the existence of long-lived FTE cells in vivo, a comparison of them further revealed that FTE cells in the fimbrial portion might give rise to FTE cells toward the proximal portion to maintain the FT epithelium. Furthermore, we separated the FT into multiple portions and cultured FTE cells from each portion in an organoid culture system we established; we found that FTE cells from the fimbrial portion were enriched with organoid-forming cells, whereas those from the proximal portion were depleted of organoid-forming cells. Collectively, our data support that the fimbrial end of the FT is enriched with FTE stem/progenitor cells. Due to the close proximity of these cells to the ovary, they (and/or their daughter cells) can be exposed to follicular fluid at the ovulatory rupture site and may even become entrapped in the ovary through the rupture site, thus providing a way for them to develop as "ovarian" cancer.

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T-1164

ZOLEDRONIC ACID GENERATES BREAST TUMOR-SUPPRESSIVE BONE MARROW HEMATOPOIETIC PROGENITOR CELLS THAT LOSE SUPPRESSIVE ACTIVITY IN RESPONSE TO G-CSF

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The bone-targeting agent zoledronic acid (ZA) increases breast cancer survival in subsets of patients when used as adjuvant therapy, but the underlying reasons for this protective effect are unknown. ZA is known to modulate the activity of osteoclasts and osteoblasts, which participate in hematopoietic stem cell niches. We demonstrate that ZA induces transient changes in numbers of hematopoietic stem cells, myeloid-biased progenitor cells, and lymphoid-biased cells in the marrow, concomitant with its known effects on bone. Importantly, we demonstrate that bone marrow cells from mice treated with a single, clinically relevant dose of ZA inhibit breast tumor outgrowth when admixed with tumor cells in vivo. Using a preclinical model of bone metastasis, we identified matched human breast cancer cell lines that were sensitive or resistant to ZA treatment and discovered that the tumor-derived cytokine G-CSF mediates resistance to the tumor suppressive effects of ZA. Interestingly, exogenous G-CSF administration was also sufficient to negate the tumor-suppressive effect of ZA. These findings provide novel evidence that hematopoietic cells play a fundamental role in mediating tumor suppressive effects of ZA and that G-CSF negates the anti-tumor activity of ZA. We are currently analyzing G-CSF levels in blood samples from stage II/III breast cancer patients (n=396) enrolled in a clinical trial for adjuvant ZA treatment. Our results suggest possibilities for capitalizing on the beneficial effects of ZA in reducing breast cancer development and progression in patients.

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T-1166

ESSENTIAL ROLE OF N6 -METHYL ADENOSINE MODIFICATION OF MRNAS IN GLIOMA STEM-LIKE CELL MAINTENANCE AND RADIORESISTANCE

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Glioma is the aggressive malignancy of glial cells with poor survival expectancy. The irradiation and adjuvant chemotherapy fail to improve the prognosis to greater extent. Major obstacle in therapy strategies is the presence of enriched population of glioma stem-like cells (GSC) which elicits resistance to drugs and leads to recurrence. Epitranscriptome alters the message conveyed by DNA code via diverse RNA modifications. The reversibility of these punctuated mRNAs is a lucrative mechanism for GSCs which portray vivid dynamics and heterogeneity. N6-methyl adenosine is one of the abundant mRNA modifications which influences various events in RNA metabolism. METTL3 (methyl transferase-like 3) catalyzes methylation of adenines of mRNA at N-6 position and its function remains unresolved in glioma. Here, we report the crucial role of METTL3-mediated m6A modification in GSC (neurosphere) maintenance and dedifferentiation of glioma cells. RNA immunoprecipitation using anti-METTL3 and anti-m6A identified SOX2, one of the glioma reprogramming factors, as a bonafide m6A target of METTL3. N6-Methyl adenosine modification of SOX2 mRNA by METTL3 enhanced its stability and exogenous overexpression of 3'UTR-less SOX2 alleviated the inhibition of neurosphere formation observed in METTL3 silenced GSCs. METTL3 binding and m6A modification in vivo required three intact METTL3/m6A sites present in the SOX2 3'UTR. Further, we found that HuR recruitment to m6A modified RNA is essential for SOX2 mRNA stabilization by METTL3. METTL3 silenced GSCs showed enhanced sensitivity to γ-irradiation and reduced DNA repair efficiency as evidenced from the accumulation of γ-H2AX. Exogenous overexpression of 3'UTR-less SOX2 in METTL3 silenced GSCs showed efficient DNA repair and also resulted in the rescue of neurosphere formation from METTL3 silencing induced radiosensitivity. GBM tumors have elevated METTL3 transcripts and silencing METTL3 in U87/GSCs inhibited tumor growth and prolonged mice survival. METTL3 transcript levels predicted poor survival in GBMs with high GSC-specific signature. Thus our study uncovers the importance of m6A modification in GSCs and reports for the first time METTL3 as a potential molecular target in GBM therapy.

T-1168

IPSC ASSAY FOR THE DIAGNOSIS OF PATIENT-SPECIFIC SENSITIVITY TO CORTICOSTEROID THERAPY

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Cancer patients who receive high-dose corticosteroid chemotherapy can develop severe bone damage, possibly leading to bone collapse and life-long disabilities. It is unclear why some patients develop osteonecrosis after intravenous corticosteroid therapy while others do not, however some studies have described genetic polymorphisms that can lead to an increased sensitivity to corticosteroids. Currently there are no effective methods of proactively screening for this sensitivity. This study evaluates the feasibility of a novel assay for detecting patient-specific chemotherapy-sensitivity in vitro. Consenting patients who have undergone corticosteroid-based chemotherapy treatment and developed osteonecrosis (n=3) underwent cytotoxicity assays. Three patients served as controls. Blood was drawn from each of the patients and PBMNC were isolated in cell preparation tubes via centrifugation. PBMNC were then reprogrammed to iPSC using Sendai virus. iPSC and iPSC-derived MSC from both groups were exposed to dexamethasone (100nM) over 5 days and 21 days, respectively, in the presence or absence of hypoxia. The cytotoxicity of dexamethasone-exposed iPSC was compared with unexposed controls using caspase activity assays. iPSC from patients who developed osteonecrosis showed a caspase activity of 37260.11±4804.71 (treated) and 20185.00±1184.95 (untreated), while iPSC of control patients showed a caspase activity of 31842.78±2910.17 (treated) and 22825.44±1632.20 (untreated). Dexamethasone treatment had a significantly higher effect on the osteonecrosis samples: Δ17075.11±5613.39 (osteonecrosis) vs. Δ9017.33±4222.93 (control) ($p=0.049$). iPSC-derived MSC showed a caspase activity of 16919.89±4316.45 (day 21) and 8332.33±1126.80 (untreated), while iPSC-MSC of control patients showed a caspase activity of 14479.78±2939.68 (day 21) and 8165.00±1031.61 (untreated). No significant difference was observed in the effect of dexamethasone between the two groups: osteonecrosis MSC (Δ8587.55±3870.11), control MSC (Δ6314.77±3878.46) ($p=0.51$). Therefore, the iPSC drug sensitivity test can be used as a non-invasive assay to predict patient specific sensitivities for cortisone treatment and related risks of developing osteonecrosis.

T-1170

ISOLATION OF METASTASIS-INITIATING CELLS IN BREAST CANCER BRAIN METASTASES

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Brain metastases are the most common malignancy of the CNS. Approximately 10-15% of patients with metastatic breast cancer develop brain metastasis (BM) with very low survival rate. The brain relapse occurrence suggests that disseminated cancer cells acquire specialized functions to reach and colonize this organ, although the biology regulating this process is fragmentary. Human metastasis-initiating cells (MICs) have not yet been identified. However, some experimental evidences suggest that MICs might be found within subpopulations of cancer stem cells (CSCs) and show capability to survive in the circulation and seed a secondary site. We isolated stem-enriching tumorspheres from breast cancer brain metastases derived from 3 patients. We analysed the clonogenic capability of these samples by methylcellulose assay, determining the clonogenic cells as percentage on the total number of seeded cells. The same cells were intracerebrally transplanted in nude mice at decreasing concentrations, and through the ELDA algorithm the MICs frequency was calculated in the derived tumors. In conclusion, we demonstrated the presence of a stem-like population in BMs secondary to breast cancer that is able to trigger tumor growth and to be serially transplanted in nude mice, and that recapitulates the original patient tumor.

Funding Source: Italian Association for Cancer Research

T-1172

TARGETING OF ROR1-DEPENDENT WNT5A-SIGNALING BY CIRMTUZUMAB: A FIRST-IN-HUMAN PHASE I TRIAL FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Cirmtuzumab is a first-in-class humanized mAb specific for ROR1, an oncoembryonic antigen found on cancer stem cells of various cancers but not on normal post-partum tissues. By binding a functional epitope in the extracellular domain of ROR1, Cirmtuzumab can block ROR1-dependent, non-canonical WNT5A-signaling. We initiated a phase 1 trial in patients (pts) with progressive, relapsed/refractory CLL, a cancer with high rates of ROR1 expression. Pts received four biweekly infusions of Cirmtuzumab at doses ranging from 0.015 to 20mg/kg. As of Jan. 2017, 24 pts have received Cirmtuzumab. Cirmtuzumab was well tolerated with no drug-related SAEs or DLTs. Anemia (19 pts), thrombocytopenia (16 pts), and neutropenia (11 pts) were the most common AEs, were primarily grade 1, and were likely disease-related. We detected significant levels of Cirmtuzumab for over 8 weeks following the last infusion and determined that it has a half-life of >24 days. Approximately 24 hours after the 1st infusion, leukemic cells of patients treated at doses > 2 mg/kg had a 70-90% inactivation of RhoA, which was observed to be active at baseline. Loss of GTPase activation also was observed for CLL cells at later time points. Clinically, after each infusion, pts typically had a transient initial increase in absolute lymphocyte count (ALC) that peaked 24 hrs after infusion, with a subsequent reduction in the ALC to levels at or below baseline. This may reflect the capacity of Cirmtuzumab to inhibit CLL-cell migration. After completing Cirmtuzumab treatment, 16 of 18 evaluable pts had stable disease and 2 had progressive disease, both of whom received low doses. Some patients had clinically meaningful (>50%) reduction in CLL-cell marrow infiltration or reduction in the ALC. Pts were also noted to have a long progression-free survival (PFS) before requiring other therapy (median 263 d, range 112-414 d). Overall, UC-961 is well-tolerated with an excellent safety profile over a range of doses and promising PK and PD. These data support further development of Cirmtuzumab as treatment for patients with cancers or cancer stem-cells that express ROR1.

CHROMATIN AND EPIGENETICS

T-1174

DNA HYDROXYMETHYLATION AND METHYLATION DYNAMICS DURING RAPID REPROGRAMMING OF SOMATIC CELLS INTO IPSC

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DNA methylation is a major epigenetic mechanism controlling cell fate decisions such as early embryo development and reprogramming into induced pluripotent cells. However, studying this process during embryo development is extremely challenging as material is limited, and cell reprogramming has the drawback that is typically very inefficient and demethylation of pluripotency genes only occurs after 2 to 3 weeks. As an alternative we recently described a highly efficient reprogramming system consisting of B cells treated with an 18h pulse of C/EBPa and subsequent exposure to the Yamanaka factors OSKM (Di Stefano et al., Nature 2016). The C/EBPa sensitized B cells reprogram into iPSCs within about 1 week and the resulting cells contribute to chimera formation. We have now taken advantage of this system to generate genome wide 5hmC and 5mC maps at nucleotide resolution during the first 4 days of reprogramming towards pluripotency. We observed a gain of 5hmC and a loss of 5mC at regulatory regions of the Oct4 and Nanog genes as early as 1 day upon OSKM activation, which is >10 times faster than in other reprogramming system described so far. Furthermore, Day4 induced cells acquire a methylation profile resembling that of ESCs. To get functional insights into the dynamics of DNA modifications we have focused our analyses at regulatory regions of the genome based on chromatin accessibility and histone modifications (ATAC-seq and H3K4me2). We observed that chromatin opening and activation typically co-occurs with loss of 5mC. Surprisingly however, we also found regions losing 5mC without detectable local chromatin opening and activation, suggesting an uncoupling of the machineries that modify DNA and histones at these regulatory regions. We also observed a dynamical complexity for the interplay between 5hmC and 5mC. While demethylation often follows hydroxymethylation, we unexpectedly also identified regions in which 5hmC is accumulated and then maintained for several days after OSKM induction, while other regions are enriched in ESCs grown under 2i conditions but not during reprogramming. In conclusion, our data suggest that the rapid DNA demethylation observed during early embryo development is recapitulated in our reprogramming experiments and that hydroxymethylation can behave as a stable epigenetic mark.

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T-1176

DEFINING THE DYNAMICS OF NUCLEAR ARCHITECTURE DURING HUMAN CARDIOMYOCYTE DIFFERENTIATION

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Congenital heart disease is a major worldwide health issue and the leading cause of birth defects and associated deaths. Many of these diseases are caused by mutations in transcription factors and epigenetic regulators. We have previously shown that many chromatin marks are dynamically regulated during the transition from pluripotent stem cells (PSC) to differentiated cardiomyocytes, leading to the identification of novel regulators of heart development. Chromosomal architecture, the structural interactions within and between chromosome strands, is emerging as means through which large-scale gene regulation occurs. We aim to identify additional regulators of differentiation by integrating nuclear architecture and chromatin accessibility to our studies of cardiomyocyte differentiation. To define genome-wide cis and trans chromosomal interactions we have performed in situ DNase Hi-C over a time course of hPSC differentiation. We identified large scale genomic domains termed TADs (topologically associated domains) across the stages of cardiogenesis. Many of these are invariant across differentiation, consistent with previous reports in other cell types. Interestingly, we identify a subset of TADs that either undergo a transition from active to repressed or the reverse, along with a subset that show changes in their TAD boundaries. Among these dynamic sites the TTN locus, one of the largest genes in the human genome and an important sarcomeric protein. At the transition from cardiac progenitor (day 5) to definitive cardiomyocyte (day 14) the TTN locus moves from a repressed to an active TAD. The topology shift is accompanied by a large increase in TTN transcription, suggesting that the change in TAD boundary promotes gene expression. Our results suggest that chromosomal

architecture plays an important role in cardiogenesis and further analysis using disease and eQTL information is ongoing to add new insights into normal development and congenital heart disease.

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T-1178

SIRT1 REGULATES EPIGENETIC STABILITY AND DIFFERENTIATION POTENTIAL OF EMBRYONIC STEM CELLS BY ANTAGONIZING DNMT3L

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Embryonic stem cell (ESC) epigenetic abnormalities hamper the utility of their therapeutic derivatives. Mechanisms responsible for cultivation and/or differentiation-mediated epigenetic alternations are unknown. Here, we show that a NAD-dependent deacetylase, Sirt1, selectively prevents abnormal DNA methylation of imprinted and germline genes in murine ESCs by antagonizing Dnmt3l. Transcriptome and DNA methylome analyses demonstrated that Sirt1 null (Sirt1-/-) ESCs repress expression of a subset of imprinted and germline genes concomitant with increased DNA methylation of regulatory elements. Sirt1-/- ESCs highly expressed Dnmt3l and knockdown partially rescued abnormal DNA methylation of the Sirt1 target genes. The Sirt1 protein suppressed transcription of Dnmt3l and physically interacted with the Dnmt3l protein, de-acetylating and de-stabilizing Dnmt3l protein. Sirt1 deficiency delayed neurogenesis and spermatogenesis. The observed differentiation delays were significantly or partially abolished by reintroduction of Sirt1 cDNA and Dnmt3l knockdown, respectively. This study sheds light on epigenetic stability of developmentally vital genes operating in ESCs.

T-1180

EPIGENETIC REGULATION OF THE METABOLIC NETWORK ACROSS CELL TYPES AND DEVELOPMENTAL PROCESSES

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Cell fate transitions are often accompanied by both significant epigenetic and metabolic changes and the connections between epigenetics and metabolism are increasingly recognized. For example, α-ketoglutarate promotes early differentiation of primed hPSCs by affecting histone demethylation. However, the reverse question, how metabolic reprogramming is regulated by epigenetic changes have not been explored. We systematically analyzed the association between chromatin state and the metabolic network across pluripotent, progenitor and adult cell types, as well as during developmental processes. We found that genes involved in retinoic acid (RA) and cyclic AMP(cAMP) metabolism are under strong but different epigenetic regulation: key genes involved in RA metabolism such as CYP26B1 are marked by both broad H3K4me3 domains (a chromatin feature of genes important for cell identity and function), and bivalent H3K4me3 and H3K27me3 domains (a chromatin feature that poised genes for activation) across a wide range of cell types, while genes in cAMP synthesis are marked by bivalent domains but not broad H3K4me3 domains. Genes encoding rate-limiting enzymes in fatty acid and glutamine metabolism (e.g., CPT1A and GLS2) are also marked by key histone modification signatures. The metabolic network is abundant with different genes that encode enzymes with the same catalytic function, and these isozymes often have different affinity for substrate or are under different post-translational modifications. Surprisingly, we observed a common phenomenon that only one of multiple genes in the isozyme family are under strong epigenetic regulation across cell types – for example, hexokinase 2 are often marked by both broad H3K4me3 and bivalent domains across a wide range of cell types, while hexokinase 1 and 3 are not. Thus, epigenetic regulation may be another layer of regulation in addition to allosteric control and phosphorylation. In summary, this study revealed that metabolic genes in second messenger and key nutrient metabolism are associated with specific chromatin signatures, and isozymes are differentially regulated by epigenetics. This provides a new tool to identify critical metabolic genes in tissue homeostasis and development by examining their epigenetic signatures.

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T-1182

IDENTIFICATION AND CHARACTERIZATION OF M6A CIRCULAR RNA EPITRANSCRIPTOMES

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This study brings together the expanding fields of RNA modifications and circular (circ) RNAs. We find that cells express thousands of m6A methylated

circRNAs, with cell-type specificity observed between human embryonic stem cells and HeLa cells. m6A-circRNAs were identified by RNA sequencing of total RNA following ribosome depletion and m6A immunoprecipitation. The presence of m6A-circRNAs was corroborated by the identification of complexes between circRNAs and YTHDF1 and YTHDF2 proteins that "read" m6A sites in mRNAs. Furthermore, m6A modifications on circRNAs depend on METTL3 and METTL14, the known m6A methyltransferase "writer" complex components. Despite sharing m6A readers and writers, m6A-circRNAs are frequently derived from exons not methylated in mRNAs. Nevertheless, m6A-mRNAs that are methylated on the same exons as those composing m6A-circRNAs exhibit less stability than other m6A-mRNAs, and this circRNA-mRNA cross-talk appears to be regulated by YTHDF2. Our results expand the m6A regulatory code through identification of the first circRNA epitranscriptome.

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ORGANOIDS

T-1184

APPROACHES FOR EX VIVO DIFFERENTIATION OF MARMOSET GERM CELLS IN INTACT TESTICULAR TISSUE

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Pre-pubertal male cancer patients undergoing chemotherapy are at a very high risk of losing their fertility. In this study we explore potential of in vitro and in vivo fertility preservation strategies like organoid culture systems and xenografting for sperm production using nonhuman primate testicular tissue. We used testis tissue from 2 newborn (half-day old) and 6 prepubertal (6 month old) marmoset monkeys to stimulate germ cell maturation and meiotic differentiation in testicular fragments by organ culture and xenografting. Influence of serum (10% knockout serum (KSR) & 10% fetal calf serum (FCS)) and hormones (500 IU\| luteinizing hormones (LH) & follicle stimulating hormone (FSH)) on seminiferous cord formation was compared in neonatal marmoset testicular fragments cultured for 4days on agar blocks, at 35°C and 5%CO_2. Histological analysis of the cultured fragment sections shows that presence of KSR in media stimulates initiation of seminiferous cord formation. Testosterone analysis shows higher testosterone production by fragments cultured in 10% KSR containing culture condition compared to other groups. Xenografting of fresh and cryopreserved prepubertal marmoset testicular tissue fragments (1-2 cm^3) in 20 intact nude male mice was performed to achieve complete spermatogenesis in marmoset grafts. Six months later grafts were explanted by sacrificing grafted mice. Higher graft survival rate was recorded amongst fresh tissue grafts compared to cryopreserved tissue grafts. Histological evaluation of spermatogenic progression in grafts shows presence of post-meiotic germ cells. In summary, for the first time we observe seminiferous cord formation in cultured neonatal testicular fragments and spermatogenic induction in marmoset testicular xenografts. This study opens novel avenues for using marmosets as suitable model for male infertility research and suggests that organoid culture and testicular grafting may become approaches for clinical applications in the future.

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T-1186

ENGINEERED HETEROTYPIC 3D CARDIAC MICROTISSUES TO PROMOTE MATURATION OF HUMAN PSC-DERIVED CARDIOMYOCYTES

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Human pluripotent stem cells (hPSCs) can yield a potentially unlimited source of cardiomyocytes (CM) for the study of cardiovascular development and disease, as well as for use in regenerative cardiac therapies. However, hPSC-derived CM exhibit fetal-like phenotypes with physiologic properties distinct from adult CM, highlighting the need for effective methods to mature hPSC-CM in vitro. We have previously shown that engineered 3D cardiac microtissues promote early nascent signs of CM maturation compared to 2D monolayer controls based on defined CM sarcomeric structures and increased sarcomere length. In this study we interrogated the effect of incorporating different stromal cells (cardiac, dermal or iPSC-derived fibroblasts, mesenchymal stromal cells) into cardiac microtissues on the phenotypic and physiologic state of CM. Engineered cardiac microtissues were generated using highly pure (>85%) hPSC-CM alone (CM tissues) or combined with stromal cells (CMF tissues) by seeding at a 3:1 ratio into microwells to form spheroidal or ring-shaped constructs. 3D microtissues maintained for 4-28 days exhibited spontaneous beating, synchronous calcium transients, and expression of cardiac markers (Nkx2.5, α-actinin, troponin T, connexin43). Single cell RNAseq could discriminate between CM (~74% of cells) and stromal cells (~18% of cells) comprising the microtissues and demonstrated that 3D heterotypic culture promoted

expression of maturation markers (MYH7, TNNI3, CKM) compared to homotypic 2D culture. Interestingly, two distinct CM subpopulations were identified within CMF tissues reflecting a spectrum of maturation states (MYH6 vs MYH7) present within the microtissues. CM and CMF microtissues could be electrically paced (0.5-3Hz) and exhibited ionotropy in response to calcium and isoproterenol. However, the presence of stromal cells increased the amplitude and decreased time-to-peak and time-to-decay in CMF calcium transients compared to CM tissues, reflecting a more mature physiologic state of heterotypic constructs compared to homotypic tissues. These results demonstrate that the combination of 3D tissue architecture and presence of stromal populations act synergistically to promote CM maturation, suggesting that complex heterotypic interactions accelerate the maturity of hPSC-CM.

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T-1188

3D BIOENGINEERED LUNG TISSUE FOR PERSONALIZED DISEASE MODELING

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Idiopathic Pulmonary Fibrosis (IPF) is a complex, rare, yet devastating disease of unknown etiology characterized by progressive and irreversible scarring of the lung tissue. Neither cell culture nor animal models fully recapitulate the progressive fibrosis and hetereogeneity of human IPF and consequently there are no effective therapies. In order to address these challenges, we generated an induced pluripotent stem cell-based, 3D model of IPF in a dish. This was accomplished by engineering a cell-hydrogel bead composite that self-organized in a rotating bioreactor to generate an organoid that resembles the architecture of the interconnecting alveolar sacs of the human distal lung. We then used several inducers of fibrosis in the organoid cultures, such as TGFb-1, to generate fibrosis in the organoid. The lung organoid formation process is modular, allowing for the use of patient-specific cells and for the generation of lung organoids in 96 and 384 well plates, making this amenable to high throughput drug screening. Using a fluorescent reporter for alpha-smooth muscle actin, one of the hallmarks of the fibrosis seen in IPF, we developed a primary screen with the ImageExpress 3D confocal device to identify compounds that reverse TGFb-1 induced fibrosis.

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T-1190

REMODELING HUMAN BARRETT'S ESOPHAGUS METAPLASIA IN A NOVEL IN VIVO ORGANOID MODEL

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Barrett's esophagus (BE) is a metaplastic abnormality in patients with Gastro-esophageal reflux disease in which the normal stratified squamous epithelium in the esophagus is replaced by columnar epithelium. BE predisposes for esophageal adenocarcinoma. One underlying mechanism of BE is that columnar stem cells residing in the esophageal mucosa (e.g., in submucosal glands) or multipotent stem cells give rise to the columnar lining. Bone Morphogenetic Proteins (BMPs) are a family of growth factors that control tissue architecture, homeostasis and stem cell differentiation. In BE, BMP4 is upregulated in columnar cells. Organoid cultures are widely used because they mimic in vivo differentiation of self-organizing stem cells and represent the perfect model to study stem cell interaction in basic and translational research. We developed an in vivo organoid model of human BE to investigate the potential to modulate the metaplastic process using an innovative anti-BMP2/4 llama-derived Dwarfbody® (DB). Endoscopic BE biopsies were implanted into immunocompromised mice intramuscularly and grown for three months with DB or control. These structures were assessed histologically and immunohistochemically (IHC) using panels of squamous, intestinal and stem cell markers. BE organoids were lined by a columnar epithelial layer containing goblet cells and recapitulated the crypt and villous regions seen within BE glands. IHC validation confirmed that the xenograft structures were of human origin and expressed markers of intestinal differentiation (CK8, CDX2 and villin). In contrast, treatment with the BMP inhibitor lead to the formation of multi-layered squamous epithelium expressing both the stem cell marker p63 and the squamous marker CK5. Tracing the fate of individual BE cell using multi-color lentiviral gene ontology vectors will allow us to understand the clonal development and the processes behind the modulation of the epithelial phenotypes. In sum, we demonstrated that inhibition of BMP2/4 in this model enhanced the amount of squamous epithelium and we are now identifying the stem cell characteristics that are at the origin of these epithelia. These pre-clinical results may be translated to the clinical setting in order improve treatment of BE and as such prevent the development of esophageal adenocarcinoma.

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T-1192

ORGANOID FORMATION FOLLOWING A PATTERN OF NEUTRAL DRIFT IN MOUSE DUODENAL STEM CELLS

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A cell competition between normal and transformed intestinal epithelial cells was observed in randomly-transformed organoid (Yamauchi et al., Sci. Rep. 2015). The radiation-induced cell competition between non-irradiated and X-irradiated stem cells has been implicated in one of the homeostasis-maintaining processes against low dose-rate radiation exposure (Otsuka and Iwasaki, J. Radiat. Res. 2015). The tracing of radiation-exposed stem cells is, however, difficult to observe until now. To clarify the radiation-induced cell competition in vitro, we assessed the organoid formation derived from intestinal stem cells in mice. First we developed the medium to form organoids high efficiency. In around 9-week-old mice, the Lgr5-EGFP-high duodenal stem cells were sorted into our adjusted medium as 1 cell per well, and they formed organoid with 25% efficiency. We made also one organoid derived from 2-color stem cells using our techniques of high-efficiency organoid forming and Lgr5-lineage tracing. Following the stem cells proliferation, disappearing the population of one-color cells from organoid was observed in the process of duodenal organoid growing. Next we analyzed the color of stem cells consisted in organoid by Flow Cytometry to assess the origin of the stem cells quantitatively. We found that non-irradiated 2-color duodenal stem cells occupied the organoid stochastically. These results suggest that the cell competition occurs in the organoid growing process and that competition between normal stem cells is like a neutral drift. The further examinations of cell competition between non-irradiated and X-irradiated stem cells are in progress to understand the radiation-induced cell competition.

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T-1194

FULLY AUTOMATED HTS-COMPATIBLE WORKFLOW FOR GENERATING HUMAN NEURAL ORGANOIDS IN TISSUE-BASED DRUG DISCOVERY

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New tools have always spurred on new insights and novel treatments. The iPS revolution coupled with emerging 3 dimensional organoid technologies bears promise to bring about the next generation of drug screening technology. So far, screening is achieved in 2D cell cultures. By letting cells self-organize in a microenvironment of their own production, scientists are able to mimic the heterogeneous cell types and complex cellular and extracellular signals of human tissues more closely. Using 3D organoids, several groups have been able to show developmental defects that were not detectable in 2D cell cultures or animal models, and more fundamental discoveries are just over the horizon. Currently, organoid protocols cannot be used for high throughput screening (HTS) approaches, since their generation involves a large amount of manual labor and are thus not scalable to the levels required for screening (10,000s to 100,000s wells). Prerequisite for such scalability is a fully automated plate-based workflow. Leading protocols rely on bulk culture in bioreactors that often produce very heterogeneous organoids incompatible with the logistics of plate-based HTS approaches at the backbone of cutting-edge screening facilities. Here, we developed a fully scalable human neural organoid workflow that is able to generate and maintain neural organoids in a fully automated liquid handling system using standard 96 well plates. Hence, our protocol is compatible with equipment already in use in screening facilities without the use of special bioreactors or specialized plastic ware. Resulting organoids show complex, self-organized structures and a large gamut of neural subtypes as evidenced by immunostaining and qPCR. Readout is facilitated by providing exactly one organoid per well. Automation also helps in reducing the inherent variability of organoid protocols by standardizing well-to-well and plate-to-plate handling. Our technology combines the power of HTS with emerging organoid biology, providing the framework for tissue-based drug discovery in the 21st century. This opens up the possibility to use HTS to help gain insight in complex multicellular processes in human neural tissues during development and disease.

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TISSUE ENGINEERING

T-1198

CRYOPROTECTION OF MESENQUIMAL STEM CELLS AND ELECTROSPUN SCAFFOLDS

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A scaffold bank with cryopreserved cells facilitates the use of biomaterials immediately after thawing for use in patients in the area of regenerative medicine. The aim of this work has been to perform cryopreservation tests with mesenquimal stem cells (MSCs) and PCL (poly(ε-caprolactone) scaffolds produced by the electrospinning technique. The PCL scaffolds and MSCs were transferred to cryovials with solution containing FBS in the following groups: G1= without freezing, G2= without cryoprotectant (CP), G3= 5% DMSO, G4= 10% DMSO, G5= 5% ethylene glycol, G6= 10% ethylene glycol, G7= 5% glycerol and G8= 10% glycerol. The tubes were frozen using a Mr Frosty container and transferred to a liquid nitrogen tank for 48 hours except for the G1, which was only maintained in cold SFB for 10min. The cells were thawed and cultivated. Treatment with CP did not alter the biomaterial in the macroscopic analysis, after freezing and thawing. Through the evaluation of the diameter of the fibers by scanning electron microscopy, no significant differences were found between the various tested CP. The mean±standard deviation of the fiber diameter of the groups G2, G3, G4, G5, G6, G7 and G8 were, respectively, 1.1±0.6, 1.3±0.7, 1.1±0.8, 1.3±0.9 and 1.3±0.9, 1.2±0.7, 1.2±0.8 µm. In terms of cell viability (MTT), after 1 day of freezing, G3, G4, G5 and G6 showed better results (with respectively mean±standard deviation absorbance 0.12±0.01, 0.11±0.01, 0.10±0.01, 0.13±0.01; $p < 0.05$) than without using CP (0.07±0.02) but not as good as the control (not freezing) (0.18±0.01). Cell viability of G7 and G8 was worse in comparison to CP (0.04±0.01, 0.01±0.01). After 6 days freezing, G4 (0.16±0.03) had no statistical difference in comparison with G1 (0.22±0.03), showing that 10% of DMSO is the best CP. The results demonstrated the adaptability of biomaterials with CP after freezing and thawing. This permits selection of the best CP to be used for freezing cells and 3D scaffolds for further use in research and application to regenerate damaged tissue.

Funding Source: CNPq, FINEP, MCTI, PROPESQ-UFRGS and Stem Cell Research Institute.

T-1200

CHARACTERIZATION OF A NEW EGF - ENRICHED COLLAGEN COATED BIOMATERIAL FOR USE AS A SKIN SUBSTITUTE

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The replacement damaged skin areas with a substitute that cannot only provide protection but also stimulate its healing is a major goal for tissue engineering. Therefore, the current study has aimed to produce and characterize an EGF enriched biomaterial using PDLLA polymer and Type 1 Collagen to be used as a scaffold for tissue engineered skin substitutes. For this proposal, scaffolds were constructed by the coaxial electrospinning technique and divided into 3 groups: 1) PDLLA, 2) PDLLA/EGF (coaxial fiber with EGF/albumin solution core) and 3) PDLLA/Collagen (a PDLLA/EGF scaffold with Type 1 collagen coating). Random fibers were obtained for all the groups, without beads. Characterization of the scaffolds was achieved by scanning electron microscopy (SEM) for morphology and pore size evaluation, confocal microscopy was used to visualize the core-shell structure, Fourier transform infrared spectroscopy (FTIR) for analysis of the presence of collagen on the fibers, and contact angle measurements (WCA) for hydrophilicity measurements. The fiber diameters for group 1 was 1.293µm ±0.320 , 1.235µm ±0.48 for group 2 and .±0.42 for group 3. All the groups showed similar pore sizes being 7.750µm, 7.410µm and 7.314µm for groups 1, 2 and 3, respectively. The core-shell relation was confirmed by confocal microscopy and FTIR analysis showed a strong peak in 1540-1660 cm-1 region in group 3 compared to groups 1 and 2 which suggests the presence of collagen in these fibers. Furthermore, the WCA for group 3 was 108,69º while for group 1 and 2 it was 118,21º and 116,58º, respectively. In conclusion, a stable core-shell fiber EGF-enriched scaffold was developed for application in skin tissue engineering, which demonstrated better hydrophilicity with collagen coating. In vitro scaffold biocompatibilities need to be performed to evaluate the potential of this new biomaterial, which could become an option for skin substitute studies.

Funding Source: CNPq, FAPERGS, FINEP and Stem Cell Research Institute.

T-1202

ACCELERATED WOUND REGENERATION BY EGF-LOADED HYALURONIC ACID BASED MICROPARTICLES

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Epidermal growth factor (EGF) is widely acknowledged to be involved in the regenerative process, but very few studies examine its effect on acute wounds such as excisions when EGF activity is constant over a period of time. This can be achieved by utilizing hydrogels and hyaluronic acid (HA) is a promising carrier for sustainable cytokine activity because it is a known ECM component that is and also biodegradable. Coupled with micro-particulates in an emulsion based approach, HA may serve to directly and stably deliver cytokines to the impaired ECM to stimulate cells for regenerative purposes. In this study, we demonstrate a cost-effective and user-friendly way to produce EGF conjugated HA microparticles which sustains EGF delivery to the impaired ECM of excisional wounds. This approach is advantageous due to its simplicity which may serve to accelerate our understanding of the wound regeneration process and relevant drug discovery.

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T-1204

UNDERSTANDING AIRWAY AND VASCULAR SMOOTH MUSCLE CELL PHENOTYPES USING REPORTER-BASED PLURIPOTENT STEM CELLS AND CELL SHEET ENGINEERING

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Angioplasty/stenting and bypass surgery have limitations as treatments for vascular occlusive disease, leading to the development of synthetic grafts and tissue-engineered blood vessels; however, immunogenicity remains a problem when autologous cell sources are unavailable. Induced pluripotent stem cells (iPSCs) provide an alternative cell source, where non-immunogenic cells of multiple lineages, including endothelial cells (ECs) and vascular smooth muscle cells (vSMCs), can be derived from patient-specific iPSCs. Although many studies have developed protocols for differentiating SMCs from iPSCs, there is no surface marker for isolating pure populations of vascular-specific SMCs. Smooth muscle actin (SMA) is a characteristic intracellular marker; however, it is also expressed in cardiomyocytes and myofibroblasts. Furthermore, SMA does not distinguish between SMCs from different anatomical locations (vascular, airway, etc.). To address these issues, we have generated a novel mouse iPSC line containing a dual-reporter (green fluorescent protein (GFP) for SMA and red fluorescent protein (DsRed) for neural/glial antigen 2 (NG2)). We differentiated this line into both ECs (using VEGF) and vSMCs (using PDGF-BB and TGF-β). ECs were isolated using co-expression of CD31/CD144, and vSMCs, which are characterized by co-expression of SMA/NG2, were isolated using our dual-reporter system. iPSC-derived SMCs expressed characteristic markers (SMA, SM22α, MHC, NG2), but with lower levels of expression compared to primary SMCs. Cell sheets were generated from iPSC-derived SMC-like populations (vascular (SMA-GFP+/NG2-DsRed+) and airway (SMA-GFP+)) using an enzyme-degradable hydrogel substrate, allowing for non-damaging release of cell sheets. The SMC cell sheets were assessed for mechanical properties using a uniaxial tensile tester as well as for ECM content and structure, specifically elastin and collagen. In addition, we have generated a human iPSC reporter line (eGFP for Acta2), and we have similarly assessed the gene expression and functional profile of the Acta2-eGFP+ cells to better understand the vSMC phenotype in the human system. With this study, we will gain a better understanding of SMC phenotypes and their functional properties.

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T-1206

AN INEXPENSIVE ENCAPSULATION METHOD FOR MESENCHYMAL STROMAL CELLS

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Cell encapsulation has the potential to revolutionize cell therapies by allowing cells to be protected from immune surveillance after transplantation into a host and the delivery of their biomolecules to the host. We developed an inexpensive, reproducible and scale-able method of encapsulating mesenchymal stromal cells (MSC). First, alginate droplets were produced by aerosolizing 1-3% alginate under nitrogen. The droplet size, quantity and dispersion pattern were assessed,

and nozzle size, alginate flow rate and concentration, and nitrogen pressures and flow rate were adjusted to obtain particles within the target size (c.a 100 microns). Next, medium containing MSCs was introduced into the alginate stream prior to aerosolizing, and capsules containing MSCs were collected. The viability of encapsulated MSCs was determined using AO/PI live/dead staining. Three bioassays were used to assess the impact of encapsulation on MSC physiology: 1) MSC growth via MTT; 2) MSC microvesicle (MV) production; 3) MSC cytokine production/release. The results are proof of concept for an inexpensive and scale-able microencapsulation method which could be adapted to a variety of cell types.

T-1208

EFFECT OF OSTEOBLAST CELL LINE-DERIVED EXTRACELLULAR MATRIX-HYBRIDIZED PEGDA SCAFFOLD ON OSTEOGENESIS OF HUMAN MESENCHYMAL STEM CELL

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Demineralized bone matrix (DBM) is useful material for bone repair, because of its osteoconductive and osteoinductive properties. Though demand of human DBM for clinical use in bone regeneration is increasing, therapeutic usage of DBM has limitation due to limited supply of human bone and distribution of medical efficacy as donor's age and state. A lot of bone repair agents have been developed but their performances were not enough to be used as alternative of DBM. To solve this problem, we focused on the ECM produced by in vitro cultured cell line. Cell line-derived ECM can be easily decellularized, sterilized and produced in controlled environment. In this work, we analyzed the effect of the ECM on human mesenchymal stem cells (hMSCs). Mouse preosteoblast cell line, MC3T3-E1 was cultured with ascorbic acid and β-glycerophosphate for 2 weeks to form thick ECM layer. And then, decellularization was conducted by simple procedure using Trypsin-EDTA and Triton X-100. We manufactured digested ECM-PEGDA hybrid sponge scaffold and hMSCs were cultured on the scaffold to analyze its osteoinductivity. We suggest that utilizing cell line-derived ECM in vitro can be an efficient tool in tissue engineering study.

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T-1210

IMPROVING THE BIOLOGICAL FUNCTION OF OVINE DECELLULARIZED HEART VALVE THROUGH THETERING OF SIGNALING PROTEINS

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It has been demonstrated that widespread clinical application of decellularized xenogeneic heart valve (DHV), as promising products for human heart valve replacement,canbelargelylimitedduetodecellularization protocol and the risk of progressive degeneration, inflammation and calcification. To overcome this limitation, after setting up decellularization protocol, the biological function of DHVs surfaces through integration of protein tethering and three-dimensional cell seeding in bioreactor have been improved. Heart valves treated with Triton X-100 and sodium deoxycholate-based protocol was completely cell free whereas preserved their biochemical and biomechanical properties and showed lack of cytotoxicity. In order to demonstrate potential clinical application of these functionalized DHVs, most important functional properties and quality parameters of surface modified DHVs including recellularization efficacy, calcification, and platelet adhesion studied both in vitro and in vivo conditions. The immobilized SDF-1α and bFGF on DHV improved significantly recellularization with endothelial progenitor cells under three-dimensional culture condition in bioreactor compared to static two-dimensional culture conditions. Cell fate analysis showed higher fibroblast-like cells and less myofibroblast-like cells in both tethered DHVs. However, SDF-DHV led to a significant enhancement of recellularization both in vitro and in vivo conditions compared to bFGF-DHV and demonstrated less inflammatory cell infiltration. Furthermore, the calcification and platelet adhesion were more decreased by SDF-DHV. Thus, integration of SDF-1α tethering and three-dimensional cell seeding in bioreactor might provide a novel and promising approach for production of functional heart valves for clinical applications.

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T-1212

CRISPR/CAS9 NUCLEASE-MEDIATED GENE KNOCK-IN IN BOVINE-INDUCED PLURIPOTENT CELLS

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Efficient and precise genetic engineering in livestock such as cattle holds great promise in agriculture and biomedicine. However, techniques that generate pluripotent stem cells, as well as reliable tools for gene targeting in livestock, are still inefficient, and thus not routinely used. Here, we report highly efficient gene targeting in the bovine genome using bovine pluripotent cells and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 nuclease. First, we generate induced pluripotent stem cells (iPSCs) from bovine somatic fibroblasts by the ectopic expression of yamanaka factors and GSK3β and MEK inhibitor (2i) treatment. We observed that these bovine iPSCs are highly similar to naïve pluripotent stem cells with regard to gene expression and developmental potential in teratomas. Moreover, CRISPR/Cas9 nuclease, which was specific for the bovine NANOG locus, showed highly efficient editing of the bovine genome in bovine iPSCs and embryos. To conclude, CRISPR/Cas9 nuclease-mediated homologous recombination targeting in bovine pluripotent cells is an efficient gene editing method that can be used to generate transgenic livestock in the future.

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T-1214

INDUCTIVE MESENCHYME FROM MOUSE AND HUMAN NEURAL CREST CELLS

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Neural crest cells (NCC) are stem cells that migrate to form multiple tissues in the head and trunk. Cranial neural crest cells migrate to the presumptive face and differentiate into multiple tissue types in the face and oral cavity. Early stage neural crest derived dental mesenchyme is vital for the genesis of teeth and supporting structures. However, molecular control for dental mesenchyme induction remains elusive. Despite tremendous progress, a translational barrier for tooth regeneration is our insufficient understanding of native pivotal signals that may be captured and utilized for the programming of somatic cells into inductive dental mesenchyme and additional craniofacial tissues. We hypothesized that the early stage condensed mesenchyme has a unique, transient gene expression profile containing required craniofacial induction signals during a short temporal period peaking at E14.5. To uncover key signals, laser capture microdissection on condensed dental mesenchyme from E14.5 and E17.5 mouse tooth germs were performed for downstream RNA sequencing. RNA Seq and enrichment analyses yielded clusters of putative genes and pathways that may play significant roles in dental mesenchyme induction. A total of 856 differentially expressed genes and 69 enriched pathways over 2 fold were discovered, with a selected set of genes verified by qPCR. To explore the programming of NCC cells towards dental mesenchyme, two enriched NCC sources were used, both mouse and a human ES cell derived population. The derived neural crest stem cells were characterized and differentiated towards mesenchyme and odontogenic lineages and showed positive for Nestin, p75, AP-2, and HNK1, in addition to forming classic neural rosette patterns. This confirmed a relatively homogenous starting population for further differentiation strategies. A selected set of growth factors, hypothesized to have an odontogenic effect; FGF8, BMP2, BMP4, confirmed a preference for proliferation or differentiation, respectively. Together, uncovering the factors necessary for proper transition from neural crest to dental mesenchyme will contribute to future craniofacial regeneration strategies and next generation patient specific cell therapies.

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ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

T-1218

STEM CELL SCIENCE AND SOCIAL JUSTICE

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As stem cell research moves toward clinical translation and therapeutic application, ethical focus must be broadened to include questions about the interface of science and society. Among these societal questions is how to conduct this research in a socially just manner, as well as how and why stem cell research ought to be used as a vehicle to advance social justice imperatives. This submission provides an overview of justice as a social and philosophical construct, and how it can be

incorporated into science discussions. This review then addresses prominent social justice challenges, especially as they relate to biomedical research and healthcare, and how stem cell research might be a mechanism to reduce the burden of these injustices. Finally, this presentation concludes with ways to structure our ethical thinking and scientific debate in order to determine if stem cell research is being pursued in a socially just manner.

T-1222

NETWORK FOR STEM CELL RESEARCHER IN KOREA

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The Korean Ministry of Science, ICT and Future Planning is in pursuit of developing stem cell platform technologies that can act as a common foundation for future bio medical technologies through the policy planning and support activation of domestic stem cell research. As part of the stem cell platform technology promotion support project, the KoNSCRT (Korea Network for Stem Cell Research & Tech-development) website was constructed in 2013. The KoNSCRT homepage is operated under Korea's representative bio policy information portal site, Bioin (http://www.bioin.or.kr), and it aims at offering a place of networking for stem cell researchers to exchange opinions and share achievements. The KoNSCRT homepage offers domestic news on stem cells, stem cell trends materials, introduction of KoNSCRT, issues recommended by experts, and KoNSCRT reports. In particular, the technology/infrastructure search menu inside the homepage shares information on technologies and materials possessed by domestic stem cell researchers to promote cooperative studies. Thus, this introduces the KoNSCRT homepage constructed for the purpose of networking promotion and information exchange among stem cell researchers.

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CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

T-1224

PRELIMINARY RESULTS OF THE HUMAN UMBILICAL CORD MESENCHYMAL STROMAL CELL (HUC-MSC) TRANSPLANTATION IN MYOCARDIAL ISCHEMIA (HUC-HEART TRIAL)

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The HUC-HEART trial (ClinicalTrials.gov Identifier: NCT02323477) is a phase 1/2, controlled, multicenter, randomized study of the intramyocardial delivery of allogeneic HUC-MSCs in chronic ischemic cardiomyopathy (CIC) patients. Three-arm study will enroll 79 patients (ages 30-80) LVEF ranging between 25-45% are randomized in a 2:1:1 pattern to receive either HUC-MSCs (group 3) or autologous bone marrow-derived mononuclear cells (BM-MNCs) (group 2) in combination with coronary arterial bypass grafting (CABG) surgery. Control patients (group 1) do not receive any cells or vehicles after CABG surgery. All patients are screened before (baseline) and at 1, 3, 6 and 12 months following CABG surgery and cell transplantation. Several clinical and laboratory tests are used to evaluate the outcome including LVEF (%) and infarct size (%) assessed by echo, MR, SPECT and PET imaging. Between mid-April 2015 and mid-January 2017, total of 44 patients were enrolled in the study; in this particular results of abstract 26 patients were presented (n=4, n=4, n=18 in groups 1, 2 and 3, respectively). The mean follow up time, so far was found as 6.3±3.4 months. One patient in group 1, 2 patients in group 2 and 1 patient in group 3 were died due to low cardiac output reasons within 1-3 months, with no direct relation to cell transplantation. Cardiac arrhythmia developed in 1 patient in group 2 and 1 patient in group 3 which were then controlled with antiarrhythmic treatment. After first six months follow-up, LVEF was found increased compared to baseline values, 1 (25%), 3 (75%) and 11 (61%) patients in groups 1, 2 and 3, respectively. Mean LVEF value changes in all patients were found as; 2% decline in group 1 (p=0.456),

7% incline in group 2 (p=0.363) and 7% incline in group 3 (p=0.015). Preliminary results represent both safety and efficacy of HUC-MSCs in CIC patients compared to controls, although the results obtained so far are limited to uncompleted data of 24 patients and in a very small number of data set. However, intramyocardial delivery of HUC-MSCs indicates a remarkable safe and beneficial following myocardial infarction.

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T-1226

HUMAN INDUCED PLURIPOTENT STEM CELLS-DERIVED EXTRACELLULAR VESICLES CARRYING SELECTED MICRO-RNAS BOOST CARDIOMYOGENIC AND ANGIOGENIC POTENTIAL OF CARDIAC STROMAL CELLS

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Extracellular vesicles (EVs) are small circular structures composed of cellular membrane and cytosolic cargo, mainly in the form of small RNAs, proteins and lipids. EVs are shed by virtually all cell types under physiological and pathological conditions and by transferring their bioactive cargo to other cells, they may influence cell fate and behavior. We have previously shown that EVs released by human induced pluripotent stem cells (hiPSCs) exert cytoprotective and stimulatory effects on human cardiac mesenchymal stromal cells (hcMSCs). In this study, we investigated efficacy of EVs isolated from hiPSCs in transferring selected proangiogenic and cardiomyogenic miRNAs (miR-1, miR-199a and miR-126) to hcMSC and their impact on cardiomyogenesis and angiogenesis. hiPSCs were genetically engineered to co-express selected miRNAs and green fluorescent protein (copGFP) by lentiviral transduction. EVs were isolated from conditioned media collected from serum-free and feeder-free cultures of hiPSCs by sequential ultracentrifugation (2000g; 100 000g x2). Expression of the transgenes was analyzed in EVs and their parental cells by real time qPCR. Following EVs transfer to hcMSC, the recipient cells were subjected to cardiomyogenic and angiogenic differentiation. Genetic modification of hiPSCs resulted in stable and constitutive expression of selected miRNAs both in cells and their EVs. Introduction of specific miRNAs in hiPSCs triggered global changes in miRNA expression pattern, particularly in case of miRNA-199a over-expression. EVs cargo was efficiently transferred to the acceptor cells – hcMSC and enhanced their differentiation towards cardiac and endothelial lineages which was confirmed by molecular analyses. This study shows the potency of hiPS-EVs in transferring micro RNAs to target cells, which epigenetically regulate gene expression changes, thus influencing cell fate. Results presented here may further be exploited in regenerative medicine to enhance proliferation and differentiation ability of tissue-residing stem cells, particularly for heart repair.

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T-1228

RENAL POSTNATAL EPIMORPHIC REGENERATION IN RATS

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Limb amputation has offered an excellent model to study regeneration biology for a long time. Cardiac apex resection in neonatal mammals has given us better understanding of cardiac regeneration. Recently, we reported the unilateral partial resection of the kidney on neonatal rats on postnatal day 1, and partial resectied kidneys bulged into the newly formed cortex from the edge of the wound with less inflammation and apoptotic cells. Total nephron segments were never totally reconstituted after resection in adult kidney. Whether the neonatal kidney can maintain the capacity for epimorphic regeneration is not elucidated yet. Our previous results surged us to ensure that the kidneys of early neonate rats might retain the capability to give rise to morphological and functional reconstitutions after serious loss of renal volume. We performed 5/6 nephrectomy, which means unilateral nephrectomy and surgical resection of 1/3 superior and inferior pole of contralateral kidney, on neonatal rats on postnatal day 1, and 8 week adult rats. In the former, complete regeneration of morphology was observed 30 days after resection, but not in latter. The regenerative processes of neonatal rats were sequentially visualized and quantified by MR imaging. The volume regrowth linearly proceeded for 1 month, and resulted in the normal renal morphology like fava beans. In the tissue staining, the regenerated neonatal kidneys, which were regrown from the margin of the resection, were constituted with every renal components one week after resection. The neonates showed that BUN/Cr returned

to normal value 2 weeks after resection. Molecular analyses demonstrated that renal developmental genes such as Notch2 and Foxd1 in regenerated kidneys were highly expressed in the nephrectomized rats, compared with non-treated control rats. Rats has the capability for epimorphic regeneration of the kidney following the resection on postnatal day 1. The phenomena not only offer a novel model in the field of regenerative nephrology, but also impact pediatric nephrology.

T-1230

KRYPTONITE FOR SUPERBUGS: MESENCHYMAL STEM CELL MODULATION IN COMBINATION WITH ANTIBIOTICS TO TREAT MULTIDRUG RESISTANT INFECTIONS

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Antimicrobial resistance is one of the greatest challenges facing the medical community today and new interventions are needed. Recent studies have shown that mesenchymal stem cells (MSC) exhibit antimicrobial activity when activated by Toll-like receptor (TLR) ligands in vitro. Previous studies in our laboratory demonstrated increased bacterial killing and wound healing in a mouse model of chronic S. aureus biofilm infection when animals were treated with MSC preactivated with a TLR 3 ligand in combination with antibiotics. We hypothesized that activated MSC could enhance the activity of conventional antibiotics in multidrug resistant infections. To test this hypothesis, MSC derived from adipose tissue of dogs were expanded in vitro and activated with TLR ligands. The effects of these cells on antimicrobial activity against multidrug resistant organisms and on production of antimicrobial peptides (AMP) were examined. These cells were tested in a bacterial killing assay with several isolates of common multidrug resistant organisms cultured from dog samples sent to the veterinary diagnostic laboratory at CSU. MSC were combined with an antibiotic to which the organism was resistant and then infected with that organism. Following incubation quantitative bacterial cultures were performed. The effects of MSC treatment in vivo were assessed utilizing a spontaneous model of multi-drug resistant infections in dogs in a veterinary teaching hospital. Immunohistochemistry and ELISA were used to assess production of antimicrobial peptides. Both activated and resting MSC produced cathelicidins. Activation of MSC with TLR ligands significantly increased production of the AMP CXCL10. MSC acted synergistically with antibiotics to inhibit bacterial growth in vitro (ANOVA test for synergy, $p < .05$ considered significant). Activation of MSC resulted in immunomodulation and increased bacterial killing and resolution of MDR infections in a spontaneous canine wound model. Treatment with activated MSC induced AMP production and enhanced antibiotic therapy in vivo in infections with multidrug resistant organisms. These results suggest that stem cell therapy using activated MSC may be an effective means to treat multidrug resistant infections which currently have very few options for treatment.

Funding Source: Shipley Foundation; Colorado Clinical and Translational Sciences Institute; NIH T32 Fellowship

GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

T-2002

GENOME-WIDE INVESTIGATION OF EPIGENETIC REPROGRAMMING IN AN IN-VITRO MODEL OF MOUSE GERMLINE DIFFERENTIATION

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Germline development is characterized by a global epigenetic reprogramming, both at the level of chromatin modifications and DNA methylation. Interestingly, epigenetic reprogramming does not occur en masse throughout the genome, but rather as a concerted sequence of events affecting different genomic regions at different times. This suggests that mechanisms must exist that direct and concert the action of the epigenetic machinery on the genome during this process. A mechanistic investigation of epigenetic reprogramming in the germline has been hampered, so far, by the fact that cells had to be sourced directly from dissected animal gonads. This has made it difficult to obtain samples large enough to apply many genomics analysis techniques and, most importantly, prevented ease of genetic manipulation and engineering. In order to overcome these hurdles, we leveraged the recent development of in-vitro models of mouse germline differentiation to promote differentiation of germ cells through and past epigenetic reprogramming stages. Our ultimate goal is to dissect the molecular mechanisms at the basis of epigenetic remodeling and transgenerational inheritance, specifically investigating how different pathways converge to ensure locus-

specific epigenetic reprogramming. We show that in-vitro differentiated germ cells faithfully recapitulate epigenetic reprogramming occurring during mammalian germ cell development. Furthermore, we are currently characterizing, at the single cell level, the transcriptome and methylome on in-vitro differentiated germ cells, in order to better identify population dynamics during the in-vitro differentiation and to investigate whether heterogeneity is present in their epigenetic status. We also present a strategy that makes use of this system to perform an optimized shRNA screen for the identification of factors that direct locus-specific epigenetic remodeling observed in mouse germ cells. The insights gathered by the screen, and the availability of a well-defined and efficient differentiation model, will allow us deploy a vast range of molecular techniques to identify and study, with unprecedented detail, the key factors governing epigenetic dynamics during this important process.

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T-2004

DYNAMIC METABOLIC CHANGES MEDIATE EMBRYONIC CELL STATE TRANSITIONS

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Cell fate specification is a tightly controlled and highly dynamic process, governed by complex molecular regulatory mechanisms. Uncovering precisely how individual cells acquire distinct identities during organismal development remains a central goal in biology. Anecdotally considered a 'bystander' effect of cell fate changes, metabolism is recently gaining appreciation as a key mediator of cell fate determination. Here, we set out to investigate the functional role(s) and impact of metabolic dynamics on cell fate decisions during early mouse embryonic development. Investigating cell state changes at single-cell-resolution during the in vitro transition of mouse embryonic stem cells (ESCs) into epiblast-like cells (EpiLCs), we uncovered extensive changes in transcript levels of key metabolic regulators. Our data support a metabolic switch from an oxidative into a highly glycolytic state as cells transition into EpiLCs and acquire developmental competence for the soma versus germ cell fate decision. Forcing cells into an oxidative state through external pharmacological manipulation sustains an ESC-like state, in the presence of potent differentiation cues. In accordance with a pluripotency-promoting effect of enhanced oxidative metabolism, likely mediated through engaging key chromatin modifying enzymes to confer a naïve epigenetic state, we go on to show that cellular energy metabolism also functionally impacts primordial germ cell (PGC) specification in vitro. Our data highlight a fundamental role for a key metabolite in the mitochondrial tricarboxylic acid (TCA) cycle in conferring a stem cell state, both in naïve mouse ESCs, but also during in vitro germ cell specification, when PGCs are diverging from a somatic fate to re-acquire a pluripotent state.

Funding Source: This research was supported by a Postdoctoral Research Fellowship from the Austrian Academy of Sciences awarded to Julia Tischler, the Human Frontier Science Program, and the Wellcome Trust.

PLURIPOTENCY

T-2006

AN ORPHAN NUCLEAR RECEPTOR GOVERNS THE NAÏVE PLURIPOTENT GENE NETWORK IN HUMAN

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The gene networks that regulate alternative pluripotent states in human remain poorly defined. In contrast to its murine counterpart, the gene regulatory network that controls self-renewal and pluripotency in the human is largely unknown. So far it has been assumed that the Pluripotency Gene Network is conserved between mammalian species. Here we show that a novel orphan nuclear receptor lies at the heart of the Pluripotency Gene Network in human. Furthermore, we show in human that this orphan nuclear receptor is essential in the induction and maintenance of naïve pluripotency. Genetic depletion of this orphan nuclear receptor leads to the collapse of the Pluripotency Gene Network, while gain of function studies, together with ChiP-seq, suggest that it regulates the expression of key pluripotency network components. We also provide novel evidence that the function of other pluripotency

network components is not fully conserved between mouse and human.

Funding Source: R.O is supported by a Sir Henry Wellcome Postdoctoral Fellowship (103060/Z/13/Z)

T-2008

ERF, A TRANSCRIPTIONAL REPRESSOR OF RAS SIGNALING, REGULATES THE ACTIVITY OF ES CELL ENHANCERS AND CONTROLS CELL DIFFERENTIATION

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RAS proteins are critical in embryonic stem (ES) cells to integrate extracellular signals and initiate commitment towards differentiation. However, ground state pluripotency is promoted by pharmacological inhibition of MEK, consistent with the prevailing view in the field that RAS/MEK/ERK signaling is largely unnecessary to maintain self-renewal and pluripotency in ES cells. Here we used N-RAS-/-; H-RAS-/-; K-RASf/f; Ubiq-CreERT2 ES cells to genetically eliminate all RAS proteins and demonstrate that RAS signaling is essential to achieve the full proliferative potential and the competence to differentiate in ES cells. Interestingly, loss of ERF, an ETS (E26 Transformation-Specific) transcriptional repressor factor negatively regulated by ERK activity, rescued the proliferative defects of RASless ES cells and the blockage in cell commitment, demonstrating that proliferation and terminal differentiation can occur in the absence of RAS proteins. ERF binds to enhancers from the pluripotent transcriptional regulatory circuit and modulates the expression of critical genes for ES cell maintenance, such as MYC. Moreover, ERF needs to be evicted from the enhancers in an ERK-dependent manner by phosporylation to initiate successful differentiation in ES cells. Our data support a model in which ERF is a key RAS downstream effector reinforcing the pluripotent ground state and integrating extrinsic stimuli to allow cell commitment in the absence of RAS signaling.

Funding Source: Research was partly funded by grants from the Spanish Ministry of Economy and Competitiveness (MINECO) (SAF2013-49147-P and SAF2016-80874-P, these projects co-financed with ERDF-EU funds) to SR.

T-2010

DERIVATION OF COMMON MARMOSET PRIMED ES CELLS UNDER HUMAN PRIMED ES CELL CURTURE CONDITION

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Currently pluripotent stem cells (PSC) are classified according to developmental stages. The earlier stage of PSC which corresponds to pre-implantation epiblasts of blastocysts is called Naïve PSC, the later stage is called Primed PSC, which contain the character of advanced development. Conventional human ES/iPS cells are usually primed state although human naïve PSC have been reported by a couple of groups including ours. Knowledge of primed PSC is gathered from human and mouse data. However, there is less information of other primate PSC although we believe those are similar to human primed PSC. Interestingly, ES cells of common marmoset which belongs to new world monkey were successfully established under LIF medium with feeder cells or no additional growth factor with feeder cells. Recently we succeeded in derivation of marmoset ES cells under bFGF which is same medium as traditional human ES cells. In this presentation, we would like to show the character of marmoset ES cells and discuss the similarity and difference between human and marmoset ES cells.

T-2012

INVESTIGATING THE ROLE OF UPF3B IN PLURIPOTENT STEM CELLS

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The central dogma of molecular biology posits that DNA, the long-term repository of the genome, is transcribed into mRNA, which is then translated into protein product. Numerous mechanisms exist to maintain the integrity of the genome, but less well-known are how the mechanisms by which the cell maintains the quality of the mRNA message relate to the regulation of gene expression, particularly during development. Nonsense-Mediated Decay (NMD) represents one system through which the eukaryotic cell maintains mRNA quality, and has been demonstrated to modulate the levels of otherwise 'normal' mRNA. NMD detects and destroys mRNA transcripts that contain premature termination codons (PTC) which would preclude translation of a complete transcript, potentially leading to the production of proteins with dominant negative effects. Research has

demonstrated that there exist multiple NMD pathways that rely upon suites of partially-overlapping proteins to carry out these crucial functions. UPF3B is one protein which functions in NMD, and mutations in this gene are strongly linked to certain kinds of intellectual disability (ID) in humans, as well as to other developmental defects. Hypothesizing that mutations in UPF3B could disrupt neural development, the Wilkinson laboratory generated induced pluripotent stem cells (iPSCs) from a patient afflicted with loss of function mutations in UPF3B and ID, as well as a matched maternal control. In so doing, the Wilkinson laboratory has demonstrated that significant differences already exist in the proliferation of patient cells relative to controls. Utilizing an EdU (a thymidine analog which labels cells in 'S' phase) incorporation assay, UPF3B mutant iPSC lines were shown to exhibit defects in their ability to proliferate, a finding that was confirmed by cell-cycle analysis. RNASeq analysis of patient lines relative to controls revealed that approximately 20% of the patients' transcriptome is differentially expressed. Subsequent direct probing of transcripts via quantitative PCR found significant differences in transcripts associated with proliferation as well as neurodevelopment. These results, in addition to other published research, suggest that UPF3B and NMD are involved in the proliferation of stem cells, which may help to explain the etiology of UPF3B ID.

T-2014

EPIGENETIC BARRIER AGAINST THE PROPAGATION OF FLUCTUATING GENE EXPRESSION IN EMBRYONIC STEM CELLS

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Although embryonic stem (ES) cells have come into use in many types of basic research, results often show the variability inherent in experimental approaches. Single-cell analyses have revealed the heterogeneity of the expression level and epigenetic status of pluripotency genes in individual ES cells. In a population of ES cells, this heterogeneity would be masked due to averaging. It remains unclear whether a population of ES cells contains this variability and what induces it. Here, we demonstrate that the expression of pluripotency gene fluctuates in a population of ES cells and that fluctuations in the expression of some pluripotency genes correlate. However, no correlation in the fluctuation of expression of Pou5f1, Zfp42 and Nanog was observed in ES cells. Correlation between Pou5f1 and Zfp42 expression fluctuations was demonstrated in ES cells containing a knockout in the NuRD component Mbd3. ES cells containing a triple knockout in the DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b showed correlation between the fluctuation expression of the Pou5f1, Zfp42 and Nanog genes. We propose that an epigenetic barrier is key to preventing the propagation of fluctuating pluripotency gene expression in ES cells.

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T-2016

FGF2 IS IMPORTANT TO SUPPORT PLURIPOTENCY OF PIG EMBRYONIC GERM CELLS DERIVED FROM FETAL GONADS

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Germ cells are alternative cell sources for deriving pluripotent stem cells. When cultured with feeder cells and adequate cytokines, migrating primordial germ cells (PGCs) can be reprogrammed into pluripotent stem cells, named embryonic germ cells (EGCs). Because EGCs possess similar features with embryonic stem cells in physiological and developmental aspects, in domestic animals, EGCs are considered as a potential tools for preclinical researches and generating transgenic animals. So, in this study, we attempted to establish and characterize pig embryonic germ cells from fetal gonads. Consequently, embryonic germ cell lines were derived from the genital ridges of a porcine dpc 30 fetus in media containing LIF, FGF2 and SCF. After establishment, this cells were cultured and stabilized in LIF or FGF2 contained media. These cell lines were maintained in both condition over an extended time period and were able to spontaneously differentiate into the three germ layers in vitro. And treating RA directly induced differentiation of EGCs into neural lineage. Interestingly, cell lines cultured in LIF or FGF2 expressed different pluripotency markers. While LIF-treated pig EGCs (LIF-pEGCs) expressed only few pluripotent markers including OCT4, SOX2 and NANOG, FGF2-treated pEGCs (FGF2-pEGCs) expressed pluripotency markers such as OCT4, SOX2, NANOG and SSEA4. Pluripotent genes were up-regulated in FGF2-pEGCs and germline markers highly expressed in LIF-pEGCs, which indicated FGF2 supplement are more efficient to supporting pluripotency of pEGCs from gonadal PGCs. In conclusion, we were able to successfully derive embryonic germ cells from genital ridges of a porcine fetus. It is verified that FGF2 signaling have important roles in reprogramming and maintaining pEGCs from fetal gonad. Pig pluripotent stem cells could be useful

candidates for preliminary studies of human disease as well as a source for generating transgenic animals.

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T-2018

O-GLCNAC IS REQUIRED FOR THE TRANSITION FROM PRIMED STATE TO NAÏVE STATE OF MOUSE PLURIPOTENT STEM CELLS

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Mouse embryonic stem cells (ESCs) are "naïve" pluripotent cells, while mouse epiblast stem cells (EpiSCs) and human ESCs are "primed" pluripotent cells. Naïve mouse ESCs can be induced to primed ESC-derived EpiSCs (ESD-EpiSCs), and primed ESD-EpiSCs can be reverted to naïve reverted ESCs (rESCs). This transition from primed to naïve states is regulated by several signal pathways. O-linked β-N-acetylglucosaminylation (O-GlcNAcylation) is a posttranslational modification in the cytoplasm and nucleus. O-GlcNAc transferase (Ogt) transfers a single N-acetylglucosamine (GlcNAc) to serine and threonine residues of nuclear and cytoplasmic proteins, whereas O-GlcNAcase (Oga) removes O-GlcNAc from these proteins. O-GlcNAcylation is competitive with phosphorylation of serine and threonine residues of nuclear and cytoplasmic proteins. Therefore, O-GlcNAc is a key factor regulating signaling pathways via the inhibition of the phosphorylation of signaling components. It has been reported that, in naïve mouse ESCs, O-GlcNAc is required for the survival and O-GlcNAc on Oct4 regulates its transcriptional activity. However, functions of O-GlcNAc in mouse primed pluripotent stem cells and in the reversion from primed to naïve states remain unclear. Therefore, here, we analyzed the function of O-GlcNAc in primed ESD-EpiSCs and reversion from primed ESD-EpiSCs to naïve rESCs. Then we found that Ogt is required for the survival of primed ESD-EpiSCs and cytosolic Oga was significantly increased during induction from naïve mouse ESCs to primed ESD-EpiSCs. Furthermore, both Ogt and Oga were required for the reversion from primed ESD-EpiSCs to naïve rESCs. These findings indicate that O-GlcNAc plays an important role in the primed state and in the reversion from primed to naïve states, and may contribute to the establishment of efficient naïve human ESCs.

T-2020

AMPK ACTIVATORS ENDOW NAÏVE PLURIPOTENCY TO MOUSE AND HUMAN PRIMED CELLS

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Previous studies reported that reversion of mouse epi-stem cells (mEpiSCs) and human embryonic pluripotent stem cells (hESCs) to naïve state usually required complicated combinations of small molecules or induction of transcriptional factors. In this study, we found that activation of AMP kinase (AMPK) with single chemical compounds reverted early-stage differentiating mouse ES cells which are similar to the post-implantation epiblast to naïve state in differentiation condition. The reverted cells showed typical naïve cell morphology and contributed to chimeric mice including germ line tissue. AMPK activators also succeeded in converting mEpiSCs to naïve state with naïve markers re-expression and Tfe3 nuclear re-localization indicating that the activation of AMPK actually reverted primed cells but not just maintained remained naïve cells. Moreover, AMPK activators reset hESCs to naïve state with pluripotent gene expression profiles and X-chromosome reactivation. Simple reversion with single compound offers AMPK as a molecular fundamental of reversion mechanisms from primed cells to naïve pluripotency. Now we are trying to identify downstream genes of AMPK pathway which are responsible for the reversion function of AMPK. This study would be a valuable clue to fully elucidate the molecular machinery for naïve pluripotency.

T-2022

ELIMINATION OF UNDIFFERENTIATED HUMAN EMBRYONIC STEM CELLS BY CARDIAC GLYCOSIDES

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An important safety concern of human pluripotent stem cells (hPSCs) is the tumorigenic risk of hPSCs, since these cells form teratomas after the in vivo injection at ectopic sites. Several thousand undifferentiated cells reside in millions to tens of millions of differentiated cells are sufficient to induce teratoma in a mouse model. Thus, it is critical to remove all residue-undifferentiated hPSCs that have teratoma potential before clinical applications using hPSC-derived cells. Digoxin and lanatoside C are both FDA-approved cardiac glycoside drugs. In this study, our data demonstrated the cytotoxic effect of digoxin and lanatoside C in human embryonic stem cells (hESCs). This phenomenon was not observed in human bone marrow mesenchymal stem cells (hBMMSCs). Most importantly, digoxin and lanatoside C did not affect stem cells differentiation ability. A similar phenomenon of cardiac glycosides was shown in the hESC-derived progeny. The viability of hESC- derived MSCs also was not affected by digoxin and lanatoside. Furthermore, in vivo experiments demonstrated that digoxin and lanatoside C prevented teratoma formation. To the best of our knowledge, this study is the first to describe the cytotoxicity effect and tumor prevention of cardiac glycosides in hESCs. Digoxin and lanatoside C are also the first FDA approved drugs that demonstrated to have cytotoxicity in undifferentiated hESCs.

T-2024

OCT6 UNIQUELY REPLACES OCT4 IN INDUCING HUMAN PLURIPOTENCY

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Somatic cells can be reprogrammed into pluripotent stem cells by ectopic expression of OCT4, SOX2, KLF4, and c-MYC. Although SOX2, KLF4, and c-MYC (SKM) can be substituted by their respective family members, OCT4 has long been considered to not be interchangeable by its octamer-binding POU family members. Through a screening with 99 candidate genes, here we have identified one of POU family members, OCT6 (also known as SCIP, TST-1, and POU3F1), to be capable of functionally replacing OCT4 and inducing pluripotency in conjunction with SKM. OCT6/SKM-mediated reprogramming works with different types of human cells, but not with mouse cells. The reprogramming process involving OCT6 is relatively inefficient and slow. These vulnerabilities are mainly due to either inefficient formation of OCT6-SOX2 heterodimers onto the canonical SOX-OCT sites or low transactivation activities of OCT6. We prove with domain-swapped chimeras that modulating either the DNA-binding propensity of OCT6 or its transactivation activity can complement its vulnerabilities and produce iPS colonies as efficient as OCT4. Our work thus provides the first evidence that OCT4 and OCT6 are functionally equivalent in induction of human pluripotency.

T-2026

HYDROGEN PEROXIDE UPREGULATED ANTIOXIDANT ENZYMES AND MAINTAINED THE PLURIPOTENT STAGE IN HUMAN IPS CELL THROUGH ENHANCING NRF2 EXPRESSION

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Tilting the balance between the reactive oxygen species (ROS) and the cellular antioxidant defense would result in oxidative stress. ROS accumulation is a key contributor to disease and cell death. Nuclear factor, erythroid 2-like 2 (Nrf2) is a major transcription factor for antioxidant defense against both endogenous and exogenous stress by increasing the expression of various antioxidant enzymes and detoxification genes. Inhibition of Nrf2 impairs both self-renewal capability of pluripotent stem cell (PSC) and antioxidant defense mechanism. ROS accumulation could be physiologically induced by hydrogen peroxide (H2O2) produced from cellular metabolism. The presence of low dose H2O2 could activate Nrf2 expression and maintain pluripotent genes in iPSC. In this study, human iPSCs generated from hMSC were investigated for the effects of H2O2-induced oxidative stress on the pluripotency, antioxidant genes expression and differentiation. The iPSCs at 80% confluence were treated with H2O2 ranging from 16 - 150 µM for 24 and 48 h. The treated cells were observed for cell morphology, viability, apoptosis and senescence. The H2O2 was not cytotoxic up to 150 µM. Glutathione producing enzymes such as glutamate-cysteine ligase catalytic subunit (GCLC), glutamate-cysteine ligase regulatory subunit (GCLM) and H2O2 catalyzing enzyme (catalase, CAT) were highly expressed in H2O2-treated iPSC at 48 h. The glutathione S-transferase P (GSTP1) and glutathione peroxidase (GPx) were upregulate in iPSC treated with 16-64 µM H2O2, but decreased when exposed to 128-150 µM H2O2. Nrf2 and pluripotent markers (Oct4, Sox2, Klf4 and Nanog) were increased after being treated with 16-128 µM H2O2 for 24 h. The presence of 64 µM H2O2 in iPSCs during spontaneous differentiation processes inhibited the development of three germ layers through Nrf2 activation. The addition of n-acetyl cysteine (NAC) or vitamin C reversed the expression of pluripotent genes as well as other effects of ROS. In summary, the exposure to ROS not only drove the expressions of GCLC, GCLM GSTP1 and GPx in the iPS cells to keep the balance of cellular redox stage, but also regulate self-renewal and differentiation in pluripotent stem cells through targeting Nrf2.

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T-2028

EVALUATION OF NAÏVE HUMAN PLURIPOTENT STEM CELL CULTURE PROTOCOLS UNDER NORMOXIA

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Although mouse embryonic and epiblast stem cells, the original 'naïve' and 'primed' pluripotent stem cells (PSCs), are generally derived and maintained at normoxia (O2 > 19%), many of the current protocols for the derivation or reversion of human pluripotent cells to a naïve state are performed under hypoxic conditions (< 5% O2). We wished to determine if human PSCs, cultured under normoxic conditions in naïve medium, could be considered truly naïve. In so doing, we also questioned what criteria could confidently be used to evaluate the naïve state and if simple assays could be determined for confirmation. We initially evaluated 4 culture protocols using 2 human embryonic stem cell lines of which only 2 protocols demonstrated compact and domed colonies, appropriate for the naïve condition, after serial passage with the best cell line and culture condition selected for further analysis. We chose to evaluate differences in the naïve versus primed conditions that have been proposed in the literature such as pluripotency marker expression, X chromosome inactivation status, TFE3 (Transcription Factor Binding to IGHM Enhancer 3) localization, cadherin expression, metabolism and methylation differences. However, in some cases, the data were indeterminate or showed no difference between 'naïve' and primed, whereas in others, the results were in fact opposite to those reported. It was unclear if these differences with the published data were due to the cell line used, the normoxic conditions or simple lab-to-lab variation. Subsequently, three cell lines were cultured using the naïve medium giving the best colony morphology in normoxia. Differences were observed between the cell lines in morphological and culture characteristics as well as in gene expression profiles. However, certain gene expression markers appeared to exhibit robust and dramatic changes in all three lines which should allow them to serve as bona fide naïve culture markers if the methylation profile confirms the naïve, globally hypomethylated state in these normoxic cultures. We

will evaluate another prominent naïve culture protocol and compare cells grown under hypoxia shortly. Here we present our findings of the metrics evaluated and propose new guides for characterization of the naïve state moving forward.

T-2030

LOCUS-SPECIFIC PROTEOMICS IN HUMAN EMBRYONIC STEM CELLS IDENTIFIES ZNF207 AS A CRITICAL REGULATOR OF OCT4 AND SELF-RENEWAL

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Cell fate during development is defined by transcription factors that act as molecular switches to activate or repress specific gene expression program. The POU transcription factor OCT4 is essential for establishing and maintaining the self-renewal and pluripotent state of human embryonic stem cells (hESCs). Level of OCT4 must be precisely regulated in hESCs as a small perturbation would lead to loss of cell identity. Although genome-wide mapping of OCT4 regulatory targets and mass spectrometry analyses has identified a few OCT4-associated proteins, the complete repertoire of potential regulatory proteins for OCT4 in hESCs is not known. Here, we combine genome editing technology, chromatin immunoprecipitation (ChIP) and mass spectrometry to investigate locus-specific proteomics at OCT4 enhancer in hESCs. We identify about 150 proteins that bind to the regulatory region of OCT4, including known regulatory proteins of OCT4 (eg., OCT4, SOX2 and SALL4). We also validate the binding of a selected set of novel proteins by ChIP analysis. The identification of known regulators as well as validation of novel proteins by individual ChIP provides strong support for the reliability of our approach. More interestingly, an enrichment analysis finds the gene ontology terms involved in transcriptional regulation are overrepresented. We then perform a functional screen of transcription factors in our list by siRNAs. Strikingly, we find ZNF207, which has never been indicated a role in hESCs, an essential and indispensable regulator for maintaining the cell identity of hESCs and for cell fate change from somatic cells to stem cells during reprogramming. Knock down ZNF207 lead to differentiation of stem cells, while forced expression of ZNF207 in fibroblast significantly increase reprogramming efficiency. Furthermore, we perform genome-wide binding and transcriptional profiling of ZNF207 and integrate it to the core transcriptional network in hESCs. In summary, we provide a generalizable strategy to identify molecular components and probe dynamic regulation at a given chromosomal location in mammalian cells. Application of this approach in hESCs enable us to identify ZNF207 as a novel regulator that play essential roles in induction and maintenance of core transcriptional program of stem cells.

PLURIPOTENT STEM CELL DIFFERENTIATION

T-2032

EFFICIENT, SCALABLE DIFFERENTIATION OF NME7AB CULTURED NAÏVE HUMAN INDUCED PLURIPOTENT STEM CELLS INTO CELLS FROM ALL THREE GERM LINEAGES

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Patient-derived induced pluripotent stem cells (iPSCs) offer exciting potential for disease modeling, regenerative medicine, and therapeutic development. In order to fully realize the potential of iPSCs, it is critical to develop culture and differentiation methodologies that result in the most biologically relevant terminal cells. We are the first company to generate human naïve state iPSCs using a single, naturally occurring human stem cell growth factor, NME7AB. Naïve stem cells have several advantages over current stem cells, called 'primed' state cells. Scientists believe that because these earlier stem cells have a "clean slate", they are more easily directed to develop into functional mature cells. One of the most exciting aspects of iPS and naïve stem cell technology is the possibility of generating differentiated cells for therapeutic regeneration. Transplantation of iPSC-derived cells has been investigated for the treatment of various conditions including Parkinson's disease, diabetes, and cardiac tissue damage. From these past studies, we've identified some of the reoccurring challenges faced by translational researchers: spontaneous differentiation, insufficient number of the target differentiated cell type due to low yield, poor cell quality and functionality, and risk of teratoma formation. NME7AB cultured naïve iPSCs do not spontaneously differentiate, have a much higher yield of directed differentiation than stem cells cultured in FGF2-based media, are of high quality and functionality. In addition, and most importantly, the cells are free of any residual pluripotent cells as a result of our unique peptide that breaks the pluripotency signal and induces and synchronizes differentiation. The result is large quantities of high quality, biologically relevant cells with no risk of teratoma formation after transplant.

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T-2034

CREATING A CARDIOMYOCYTE PIPELINE FOR GENE EDITED HUMAN IPSCS

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The Allen Institute for Cell Science (AICS) is creating an open source collection of fluorescently tagged human iPSC lines to model cell organization and dynamics of stem cells both in the undifferentiated and differentiated states. We differentiate the fluorescently tagged iPSC lines into cardiomyocytes using an established protocol known to robustly differentiate multiple human ES and iPS cell lines in a relatively short time frame. Additionally, understanding the organization and activities of cardiomyocytes in greater detail may lead to advances in the development of better disease models, therapies, and regenerative medicine. Using the WTC human iPSC line and the CRISPR/Cas9 system we have fluorescently tagged 15 target genes representing key cellular organelles. We plan to study the changes in localization and organization of these organelles as the stem cell differentiates into cardiomyocytes using live fluorescent cell imaging. Differentiation into cardiomyocytes serves as an important quality control criterion for our gene editing efforts. Therefore, gene edited cells identified from genomic screening for precise editing and subjected to various quality control criteria including their ability to differentiate into cardiomyocytes. Here we present our differentiation methods and the quantitative and qualitative assays employed to determine the efficacy of differentiation including myofibril contraction, cardiomyocyte marker expression, and supporting transcriptome profiling by RNAseq. Additionally, we confirm the localization of cardiomyocyte markers such as Troponin T to the myofibrils in differentiated cells using image-based assays. In experiments initiated to date we have successfully differentiated multiple gene edited iPSC lines representing 12 major cellular structures, 2 of which are specific to cardiomyocytes (ssTNNi1 and ACTN2). Some of the gene edited structures include focal adhesions; actin and microtubule cytoskeleton, mitochondria, nuclear envelope, desmosomes, and endoplasmic reticulum. We are currently working towards developing optimized methodologies for scalable and efficient production of cardiomyocytes to support our imaging pipeline.

T-2036

DISCOVERY OF TISSUE-SPECIFIC SMALL ORF ENCODED PEPTIDES USING PLURIPOTENT STEM CELL DIFFERENTIATION

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The existence and function of small (less than 100 amino acids) peptides encoded by small ORFs (smORFs) has become an area of intense interest in recent years. It is possible that thousands of these functional peptides have been overlooked, primarily due to difficulties in discerning functional smORFs from the millions of untranslated, non-functional smORFs. Several functional smORF-encoded peptides (SEPs) have been highlighted in recent publications, supporting the notion that many SEPs are yet to be discovered. Previously published proteogenomics efforts have used RNA-sequencing and proteomics to identify SEPs expressed by immortalized cell lines. These studies have detected hundreds of SEPs expressed in human cells. Our study complements these previous efforts by providing evidence for tissue-specific expression of SEPs, and functional annotation of a subset of these peptides. To identify tissue-specific SEPs in an empirical fashion, we have performed a proteogenomics experiment using cells derived from directed differentiation of human pluripotent stem cells. Specifically, we have studied SEP expression in white and brown adipocytes, hepatocyte-like cells, monocytes, endothelial and vascular smooth muscle cells, and pluripotent stem cells. Various bioinformatic analyses have been applied to the resultant peptide database, including conservation profiling, structural motif identification, homology modeling, signal peptide prediction, and comparison of tissue-specific expression of these SEPs with public RNA expression databases. SEPs of the highest interest were encoded by LNCRNA and anti-sense RNA genes – which may now be re-classified as coding genes. We have performed high-content microscopy and arrayed CRISPR-Cas knockout screening to functionally annotate newly identified SEPs, resulting in a panel of high-priority peptides for future characterization in thorough mechanistic studies.

Future studies will include profiling many cell types derived from hPSCs, in vivo and in vitro characterization of SEP candidate function, and testing for SEP secretion, in the hope that functional peptide hormones will be identified. This work will facilitate complete annotation of the smORF catalogue in the human genome, revealing the SEP constituents of cellular function.

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T-2038

TEAD4 -/- IMPAIRS HUMAN TROPHECTODERM DIFFERENTIATION IN VITRO

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TEA domain family member 4 (TEAD4) is a crucial transcription factor in murine trophectoderm specification and trophoblast progenitor self-renewal. However, few studies were reported in human trophoblasts. We first generated TEAD4-/- human embryonic stem cell (hESC) line using CRISPR/Cas9, with no alteration in pluripotency and self-renewal, based on pluripotent markers expression, embryonic body formation and teratoma formation. When differentiated towards trophectoderm lineage in the defined medium containing bone morphogenetic protein 4(BMP4) and small molecules, TEAD4-/- cells showed significant decreased in formation of syncytiotrophoblast (STB)-and extravillous trophoblast (EVT)-like cells, based on markers expression, hormone secretion, and invasive ability compared with wild-type cells. Furthermore, overexpression of TEAD4 in TEAD4-/- hESCs rescued the ability to differentiate to STB-and EVT-like cells. Collectively, our data suggest that TEAD4 plays an essential role in human trophectoderm cells differentiation in vitro, which might indicate its crucial function in vivo. These results will provide evidence to get a better understanding of how trophoblast stem/progenitor cells differentiate during placental development and reveal the molecular mechanisms underneath.

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T-2040

CHROMOSOMAL ABNORMALITIES IN RESIDUAL UNDIFFERENTIATED STEM CELLS

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Residual undifferentiated stem cells (rSC) remain a significant risk to the clinical translation of pluripotent stem cells (hPSC) due to their ability to form tumors if accidentally transplanted in vivo. Currently little is known about the mechanisms that lay at the basis for their formation, and identifying such mechanisms may lead to improved methods for their removal, or improved differentiation protocols into a desired cell type. We previously observed a correlation between the presence of chromosomal aberrations and a more frequent formation of rSC in a colony formation assay, whereby differentiated cells were replated under pluripotent stem cell growth conditions and the pluripotent phenotype was rescued. Additionally, we found 20q21.11 duplications in some of the rSC sublines not seen in the original population. We therefore hypothesized that chromosomal aberrations commonly found in hPSC may lay at the basis for this phenotype, and identification of these abnormalities may lead to a better understanding of the mechanisms for their loss of differentiation capacity. As a result of the genetic mosaicism present in hPSC, commonly used karyotyping techniques will fail to detect low level abnormalities, while simultaneously creating a bias towards those mutations which offer a survival advantage to the cells. To overcome this inherent obstacle, we utilized single cell analysis in order to better study this phenomenon. We differentiated our in-house derived human embryonic stem cell (hESC) line for 18 days towards hepatocyte like cells. rSC were subsequently isolated by a miRNA switch technique utilizing MicroRNA-302a-5p, whereby all non rSC were eliminated from culture, increasing the purification yield relative to antibody based FACS sorting. Then, using the Fluidigm C1 single cell DNA amplification system, along with the NextSeq DNA sequencing platform, we shallow sequenced up to 96 single rSCs and report on the CNVs found. Furthermore, we compared the CNVs found in the rSC to those seen in both the undifferentiated starting population, and the final hepatocyte like cells in order to further specify CNVs associated with the rSC state. From this list of CNVs, we aim to generate a list of targets for further analysis as driver genes in the future.

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T-2042

NOX4 NADPH OXIDASE IS A KEY REGULATOR OF INDUCED PLURIPOTENT STEM CELL DIFFERENTIATION TO ENDOTHELIAL CELLS

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Induced pluripotent stem (iPS) cell-derived endothelial cells (ECs), which display characteristic morphology and marked vasoreparative capacity both in vitro and in vivo, hold clear potential for therapeutic angiogenesis in ischaemic disease. However, the precise mechanisms underlying their regulation are not known. It is well established that oxidative stress and reactive oxygen species (ROS), which are central features of ischaemic disease, are important regulators of both EC and stem cell biology, with recent evidence specifically highlighting NADPH oxidases. Here, we investigated the specific role of NOX4, which is the most highly expressed NADPH oxidase isoform in mouse iPS (miPS) cells, in their differentiation to ECs and maintenance of cell phenotype. miPS were cultured in standard endothelial growth conditions, including VEGF and collagen-IV, and differentiated to ECs for 8 days. During the differentiation process NOX4 was genetically manipulated and key EC marker expression assessed as a measure of efficiency. Our data indicate that NOX4 (mRNA and protein) is significantly and progressively induced during differentiation of miPS to miPS-ECs, suggesting an important role in this setting. Indeed, following NOX4 overexpression (plasmid transfection) endothelial progenitor markers e.g. CD144, were significantly increased vs control cells (empty vector), highlighting NOX4 as a likely key target for promoting EC differentiation efficiency. Interestingly, while endogenous ROS actions mediated by increased NOX4 activity enhanced miPS-EC differentiation, exogenous ROS induced by phorbol 12-myristate 13-acetate (PMA; 100nM for 6 days), a PKC pathway activator, inhibited this process, highlighting differential effects of ROS signalling in these cells. The precise mechanisms through which ROS may regulate miPS-EC differentiation remain unknown. Therefore, detailed investigation of the specific influence of NOX4-derived ROS on miPS differentiation through e.g. extensive genomic/proteomic studies is critical in order to identify key ROS-induced remodeling events. Nevertheless, these data highlight NOX4 NADPH oxidase as an important mediator of miPS-ECs which may have significant implications for their potential therapeutic application for ischaemic disease.

Funding Source: This work was supported by the British Heart Foundation (FS/14/35/30813)

T-2044

HUMAN PLACENTA-DERIVED EXTRACELLULAR MATRIX HYDROGEL SUPPORTS DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TOWARDS HEPATOCYTES

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Hepatocytes derived from human induced pluripotent stem cells (hiPSCs) have been considered to address the shortage of primary human hepatocytes for therapeutic needs. There are a number of protocols available to induce iPSC differentiation into hepatocytes on animal-derived matrices such as Rat Tail Collagen I and Matrigel. However, the animal origin of these substrates has huge limitation when considering translation of hiPSC derivatives to the clinic. The present study evaluated the use of human placenta-derived matrix (hpECM) hydrogel to support hepatocyte differentiation of hiPSCs. Hepatic differentiation was initiated by treating hiPSCs in suspension with Activin A before transferring cells for adherent culture on hpECM hydrogel, Rat Tail Collagen I or Matrigel. After cell attachment on each matrix, maturation was induced with stimulation from hepatocyte growth factor (HGF), dexamethasone, and Oncostatin M (OSM) for one passage. The total differentiated cell population was then expanded for one additional passage won their respective matrices. hiPSC-derived hepatocytes were identified by morphological observation and hepatocyte-specific marker expressions through quantitative test methods. hpECM supports hepatic differentiation and expansion at levels comparable to differentiations performed on Rat Tail Collagen I and Matrigel. Animal-free reagents are essential for hiPSC-based technologies in translational research. hpECM can be considered as a suitable substrate for completely humanized hiPSC derived hepatocyte culture to prevent potential risks and shortcomings of xenogeneic materials. Additionally, hpECM may also provide a valuable tool for the development of hiPSC derived in vitro screening platforms or the successful formation of 3-dimensional cell culture environments currently under investigation.

T-2046

INCREASED P53 ACTIVITY CAUSES LOSS OF CARDIOMYOCYTES DURING CARDIAC DIFFERENTIATION OF HUMAN IPSCS

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Activation of the transcription factor p53 within cancer cells is a well characterized pathway, whereas the effects of p53 activation during development remain unexplored. Previous research has indicated that increased levels of p53 protein during key murine developmental stages cause defects in a number of embryonic tissues, including the heart. These findings were confirmed in several different mouse models, but p53 activation in a human system of cardiovascular development is not available. Our lab utilizes induced pluripotent stem cells (iPS cells) derived from patients with congenital heart defects and their parents. These iPS cells undergo an established protocol of cardiac differentiation that allows us to modulate signaling pathways that would occur during normal human heart development. Pharmacological modulation of p53 protein levels with the Mdm2 inhibitor Nutlin3a for 24 hours during the cardiac progenitor stage showed a sizeable loss of cardiomyocytes and reduced expression of cardiac markers. Activation of the p53 signaling pathway in cardiac progenitors therefore induces early cell cycle arrest and apoptosis that reduces the number of cardiomyocytes formed during heart development. These results hold direct application to the study of human congenital heart defects that may be due to cardiomyocyte loss. Further use of human iPS cells will allow us to answer whether cardiac progenitors and iPS cells respond differently to p53 activation than a more mature cardiomyocyte.

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T-2048

OVEREXPRESSION OF MICRORNA-30C INHIBITS NEURAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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MicroRNAs (miRNAs) have been demonstrated to be essential for the neural development. During the neural differentiation, miRNAs play a regulatory role in embryonic stem cells by imperfect base-pairing binding to complementary sequences in the 3'-UTR of target mRNA transcripts. In our previous study that miRNA-30c played a regulatory role in self-renewal and neural differentiation in rat C6 glioma cells; however, the function of miRNA-30c in the neural development of the human embryonic stem cells (hESC) is unclear. In the present study, a human embryonic stem cell line H9 was transfected with a miRNA-30c oligos and then cells were induced and differentiated to neural cells with N2/B27 medium. The cells were analyzed for various markers including pluripotent and neural differentiation cells markers by western blotting and immunocytochemistry. MiRNA RT-PCR was performed to determine the expression of miRNAs at all selected time points after neural induction. Our findings showed that after induction, hESC H9 cells differentiated into heterogeneous pools of neural cells containing neurons, astrocytes, and oligodendrocytes. MiRNA-30c overexpression could suppress the expression of neuronal marker beta III-tubulin. This study provided a way for the further identification of novel targets of miRNA-30c, which may elucidate the mechanisms of miRNAs for the neural differentiation in hESC.

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T-2050

COMBINATORIAL ANALYSIS OF DEVELOPMENTAL CUES TO EFFICIENTLY CONVERT HUMAN PLURIPOTENT INTO SUBTYPES OF MOTONEURONS

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Specification of cell identity during development depends on exposure of cells to sequences of extrinsic cues delivered at precise times and concentrations. Identification of combinations of patterning molecules that control cell fate is essential for the effective use of human pluripotent stem cells (hPSCs) for basic and translational studies. We recently described a scalable, automated approach to systematically test the combinatorial actions of small molecules for the targeted differentiation of hPSCs. Applied to the generation of neuronal subtypes, this analysis revealed an unappreciated role for canonical Wnt signaling in specifying motor neuron diversity from hPSCs and allowed us to define rapid (14 days), efficient procedures to generate spinal and cranial motor neurons as well as spinal interneurons and sensory neurons. Our systematic approach to improving hPSC-targeted differentiation should facilitate disease modeling studies and drug screening assays. We are now developing a similar approach to better understand and efficiently recapitulate the diversity of MNs identity in vitro.

T-2052

COMPARISON IN CARTILAGE DIFFERENTIATION OF MESENCHYMAL STEM CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS AND BONE MARROW

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Human mesenchymal stem cells (MSCs) represent one of the most promising stem cells for regenerative medicine, which have been most widely used in various phases of clinical applications. However, the disadvantages in limited life-span with primary MSCs greatly hamper their use in basic research and clinic application. Primary MSCs usually undergoing senescence at around passage 10. Since its derivation in 2006, iPSCs have been considered as a possible avenue to achieve an ideal patient-specific cell source for regenerative medicine. Although there are some reports on human pluripotent stem cell-derived mesenchymal stem cells (hPSC-MSCs), the use of feeder or serum greatly compromises their clinical application. So far, there is no chemically defined, step-wise protocol to derive MSCs from human pluripotent stem cells. Using our novel step-wise and chemically-defined system, hPSC-MSCs were efficiently generated as high as up to 97.2% of CD73+ cells. Our novel protocol fully recapitulates the major developmental stages of MSCs, including primitive streak, lateral plate mesoderm, specification and cartilage formation of MSCs. Compared with bone marrow derived MSC (BMSCs), hPSC-MSCs can maintain the MSC morphology at passage 40. Considering unlimited self-renewal of hPSCs and much longer life span of hPSC-MSCs, therefore large quantity of hPSC-MSCs can be generated to overcome the limitation with primary BMSCs in cell number. hPSC-MSCs lose their pluripotent-associated markers and displayed similar morphology and surface antigen profile to BMSCs, showing negative for CD14, CD34, CD45, but positive for CD29, CD44, CD49b, CD73, CD90, CD105, CD151 and CD166. Correlation coefficient in transcriptome between hPSC-MSCs and primary BMSCs was very high by microarray. hPSC-MSCs have the ability to differentiate into chondrogenic as well as osteogenic, and adipogenic lineages in vitro. Most importantly, hPSCs-MSCs were able to fully repair cartilage defects after transplant into cartilage defects in rat model, there is similar histological grading score between hPSC-MSCs and BMSCs. Moreover, hPSC-MSCs are confirmed to participate in cartilage regeneration in the neo-tissue. These findings demonstrate that hPSCs can offer a functional patient-specific MSC source for personalized regenerative medicine.

T-2054

MODELLING NEURAL CREST STEM CELL MAINTANANCE, MELANOCYTIC SPECIFICATION AND MELANOMA INITIATION USING PLURIPOTENT STEM CELLS

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Recent attention has been paid to the development of the neural crest (NC), a transient embryonic stem cell population that originates during the process of neurulation in the dorsal portion of the vertebrate neural tube. Notably, these cells undergo an epithelial-to-mesenchymal transition (EMT), delaminate, migrate widely throughout the embryo and colonize different niches where they differentiate into several cell types. In fact NC cells can give rise to cells of mesenchymal, neuronal, secretory and pigmented identity. Melanoma is a cancer that originates from NC-derived melanocytes of the skin. Despite its highly heterogeneous nature, a specific subpopulation of melanoma cells has been shown to express NC stem cell (NCSC) markers, which were associated with tumor initiation and progression. It is therefore conceivable that fundamental mechanisms regulating NC during development may be reused for melanoma formation and growth. Yet, the molecular circuitry governing NCSC maintenance and differentiation remains largely unknown. A growing body of evidence suggests that stem cells display unique metabolic signatures and that metabolic shifts may act as a leading cause for cell fate changes. Based on these observations, the main objectives of this research project are to investigate the metabolic networks controlling NCSCs and to determine potential changes linked or even responsible for the melanocytic specification and/or related to melanoma initiation. To achieve these goals, we have generated human embryonic stem cell-derived NC cells and are currently differentiating these cells into the melanocytic lineage.

T-2056

FK866 COMPROMISES MITOCHONDRIAL METABOLISM IN IPS DERIVED NEURAL CREST CELLS

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The neural crest (NC) is an embryonic stem/progenitor cell population that generates a diverse array of cell lineages. In this study, we demonstrate the importance of the oxidized form of cellular nicotinamide adenine dinucleotide (NAD(+)) and its effect on mitochondrial activity as a pivotal metabolic switch to modulate iPS derived NCCs. We measured intracellular NAD levels of iPS cells and iPS-NCCs by high performance liquid chromatography (HPLC; Shimadzu). We also measured NAD concentration changes by the effect of the specific NAMPT inhibitor, FK866. On the other hand, we added the NAD-related intermediate metabolite, nicotinamide mononucleotide (NMN) in order to increase cellular NAD level. After NAD concentration was measured, we then evaluated oxygen consumption rate (OCR) in iPSC-NCCs by using a metabolic extracellular flux analyzer (Seahorse: XF24). After treatment with FK866 10nM for 24 hours in NCCs, NAD levels resulted in a significant reduction. Flux analyzer analysis revealed that FK866 treatment did not affect basal OCR or ECAR, however, it reduced the maximal OCR after FCCP (mitochondrial inhibitor) treatment. In contrast, treatment with NMN significantly increased NAD concentration in corneal endothelial cells. Metabolic analysis by flux analyzer revealed an increase in basal OCR as well as the maximal OCR after FCCP treatment in NMN treatment group. In conclusion, we found that hNCCs are sensitive to changes in NAD+ levels accordingly alter their metabolism especially upon mitochondrial electron transport system.

T-2058

LARGE SCALE HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION INTO CARDIOMYOCYTES IN STIRRED TANK BIOREACTORS

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Novel regenerative therapies as well as drug discovery and safety assays require large amounts of human cardiomyocytes. We have shown that cardiomyocytes can be generated from human pluripotent stem cells (hPSC) cultivated and differentiated as free floating aggregates in controlled stirred tank bioreactors (published by Kempf et al. in 2014). This bioreactor type facilitates process up-scaling and enables monitoring and control of process parameters such as pH, dissolved oxygen (DO) and feeding strategies. However, currently applied media for cardiac differentiation have limitations such as high costs and the presence of bovine serum albumin. Therefore, a more cost-efficient, chemically defined and xeno-free protocol has to be developed that is suitable for large scale suspension cultures. In this study, hPSC aggregates were generated in 150 ml scale stirred tank bioreactors. After 48 h of cultivation, 20 ml aliquots were transferred into Erlenmeyer flasks and placed on orbital shakers. This strategy allows parallel screening of several culture media modulations and concentrations of supplemental compounds including the chemical WNT pathway modulators CHIR99021 and IWP-2. This approach also allows the analysis of bioreactor vs. flask-specific culture parameters. When differentiating hiPSCs for 10 d in the medium CDM3 published by Burridge et al. in 2014, highly robust induction of beating aggregates was obtained consisting of >90% alpha myosin heavy chain positive cells revealed by flow cytometry. This chemically defined, xeno-free differentiation protocol allowed the generation of >10 Mio cardiomyocytes in 20 ml culture volume in a single Erlenmeyer flask. However, applying this protocol in impeller-stirred bioreactors resulted in decreased differentiation efficiencies of less than 75% cardiomyocytes whereas parallel controls in flasks remained at >90%. This suggests that hydrodynamic and/or physiological parameters can alter the differentiation outcome despite the use of identical hPSC aggregate suspension to initiate differentiation. The presented work investigates crucial parameters such as pH, DO and agitation rate to conclude optimal conditions and potential pitfalls for upscaling hPSC-cardiac differentiation towards industry- and therapy-compliant scale and cell quality.

T-2060

IN-VITRO ACQUIRED POSITIONAL INFORMATION AFFECTS INTEGRATION OF ESC-DERIVED NEURAL PRECURSORS TRANSPLANTED IN ADULT MOUSE BRAIN

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In the last two decades, several groups have been pursuing the idea that transplanted neural cells can reconstruct the damaged neural circuitry and promote

recovery of the injured central nervous system. Indeed, previous reports showed that the presence of a lesion greatly improves integration and survival of fetal neurons transplanted in the adult brain. Moreover, key factors for the effectiveness of the transplanted cells are their positional identity and their ability to find the correct targets. In this regard, homotopic transplantation experiments showed appropriate topographic distribution of the efferents from grafted fetal neural tissue. However, it is unclear to what extent the positional information cells acquired in-vitro may influence their ability to integrate and to find the appropriate targets in the host brain. Here, we compare integration and projection patterns of intracerebrally grafted mESC-derived neurons with cortical or hippocampal molecular identity. Firstly, we show that double inhibition of the endogenous WNT and BMP signalling during mESC differentiation induces acquisition of cortical identity, while timed activation of WNT signalling promotes hippocampal fate. The identity of mESC-derived neurons was further confirmed comparing their global gene expression profiles to that of embryonic mouse cortex and hippocampus. Secondly, we demonstrate that the positional identity acquired in-vitro by mESCs strongly influence their ability to integrate and extend far-reaching projections when grafted in the adult mouse brain. In fact, mESC-derived neurons with hippocampal identity transplanted in the dentate gyrus (DG) of adult mice send efferents toward canonical target regions of the DG, such as CA3 and entorhinal cortex; while mESC-derived cortical neurons show very few projections when grafted into DG or intact motor cortex. Nonetheless, when transplanted in the motor cortex following photothrombotic ischemia, mESC-derived cortical neurons integrate in the host circuitry, send projections toward different canonical motor cortical targets, such as striatum and internal capsule, and promote functional motor recovery of the controlateral limb.

T-2062

TRIP-TRAP INTERACTION MEDIATE THE SKELETAL MESENCHYMAL STEM CELL PROFILERATION IN FRACTION HEALING

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Bone remodeling is coupled by osteoblasts and osteoclasts, which are responsible for bone formation and resorption respectively. In general, osteocytes sense the bone damage/fracture and induce the osteoclastogenesis and bone resorption, followed by mineralized tissue deposition by osteoblasts. The mechanism of osteoblast activation at the resorption pit is largely unknown. Bone resorption pit is marked by the Tartrate Resistant Acid Phosphatase (TRAP), which is secreted by osteoclast to resorb the mineralized tissue. Using TRAP as a bait, we conducted the phage display high-throughput screening by osteoblast ORF phage library and identified transforming growth factor-beta receptor-interacting protein (TRIP-1), a high affinity protein that expressed by a subpopulation of bone marrow cells (< 10% by immunohistochemistry). TRAP-TRIP interaction was confirmed by ELISA and far-westernblotting. In vitro, TRIP-1 positive cells showed a strong multi-lineage (osteogenesis, chondrogenesis, adipogenesis) differentiation potential. In vivo, TRIP-1 cells distributed in the endosteum and periosteum of mouse long bone. In the developing long bone, the mature cartilage is absorbed by TRAP positive chondroclasts. A large number of TRIP-1 positive cells were also observed surrounding the growth plate. In the mouse femur fracture model, the TRAP positive cells, including mature macrophages and osteoclasts accumulate at the fracture gap from day 1 to day 7. Interestingly, TRIP-1 positive cells were also proliferative (positive for Ki-67) and gradually became a dominant population in the fracture callus. When the fracture site was infused with lenti-virus expressing siRNA against TRIP-1, the callus formation was significantly delayed. Similarly, relatively lower number of TRIP-1 positive cells were observed in the long bone of aging mice (2 years old) than in young adult mice. In vitro, bone marrow cells overexpressing TRIP-1 showed a high migratory activity toward TRAP in the Boyden chamber assay with increased expression of osteogenic markers, while cells knocking down TRIP-1 by siRNA showed reduced migratory activity by TRAP and low osteogenic maker expression. In conclusion, TRIP-1 is a putative marker of stem/progenitor cells that are recruited to the bone fracture site by TRAP-TRIP interaction.

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T-2064

GENERATION OF NKX2.1/P63 AND NKX2.1/CCSP KNOCKIN HUMAN INDUCED PLURIPOTENT STEM CELL REPORTER LINES FOR MONITORING GENERATION OF RESPIRATORY CELLS

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One promising option to cure hereditary pulmonary diseases like cystic fibrosis might be a cell replacement therapy comprising the generation of patient specific autologous induced pluripotent stem cells (iPSCs), followed by the correction of the underlying genetic mutation, in vitro differentiation into the needed lung epithelial cell type and replacement of the endogenous diseased cells. For long term restoration, most likely gene-corrected lung stem and progenitor cells like basal and club cells will be needed. A prerequisite of this strategy is the development of efficient and robust protocols for the generation of basal and club cells from human iPSCs (hiPSCs). Reporter cell lines serve as ideal tools for the establishment of the protocols and allow the enrichment of the desired cell types. The aim of the present study was the generation of two hiPSC double transgenic reporter lines targeting the NKX2.1 (NK2 homeobox 1; first marker of lung epithelial cells) and p63 (tumor protein p63; basal cell marker) locus as well as the NKX2.1 and CCSP (club cell secretory protein; club cell marker) locus. Therefore, we designed one targeting vector for a non-disruptive integration of an eGFP coding sequence into the NKX2.1 locus. By making use of transcription activator-like effector nucleases (TALENs) technology we successfully generated a clone that correctly integrated the targeting vector, without carrying any additional integrations of the targeting vector (confirmed by Southern Blot). The generated hiPSC-NKX2.1 line served as the basis for the generation of the double transgenic lines. The p63 and CCSP targeting vectors are comprised of a Venus (nuclear localized) coding sequence and a Neomycin selection cassette under control of the targeted endogenous promoter. These targeting vectors and the use of site specific TALENs resulted in the generation of two hiPSC-NKX2.1-p63 lines and two hiPSC-NKX2.1-CCSP lines. Southern Blot confirmed no additional integration of the targeting vectors into the genome. These established hiPSC-NKX2.1-p63 and hiPSC-NKX2.1-CCSP reporter lines represent excellent tools for the improvement of protocols for the differentiation of hiPSCs into basal and club cells and enables their enrichment which is indispensable for further in vitro and in vivo studies.

T-2066

TISSUE SPECIFIC LAMININS GENERATE AUTHENTIC AND CLINICALLY COMPLIANT CELLS

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The lack of defined, xeno-free, robust methods for efficient expansion and specialization of human pluripotent stem cells (hPSC) towards different cell types has hindered both the advancement of basic research and the translation into clinical settings. The expression and composition of the basement membrane proteins are essential for embryonic morphogenesis and adult tissue functions. Laminins influence adhesion, differentiation, migration, phenotypic stability, anoikis resistance and functionality of all cells associated to it. Laminins are the only basement membrane proteins that are tissue specific and with the use of the specific combination of xeno-free and defined human recombinant laminins, the natural environment for each specific cell type can be created, generating high quality cell with homogenous phenotypes. Laminin-521 (LN-521) is a protein naturally expressed by human PSCs and is a critical factor of the pluripotent stem cell niche. Human PSCs on Ln-521 grow as a homogenous monolayer without any abnormal genetic aberrations and with maintained expression of pluripotency markers. By using different laminins, differentiation of LN-521 cultured hPSCs can be made robust, reliable and with increased efficiency, for example: 1) dopaminergic progenitors cultured on laminin-111 (LN-111), result in an increased yield of >40 times compared to standard EB-based protocols. The GMP-compatible LN-111 based protocol gives a very homogenous population of cells predicted to have a good graft outcome. Due to the high yield, the cost of reagent per transplant is low. 2) hESC-derived RPE cells cultured on LN-521 exhibit native characteristics including morphology, pigmentation, marker expression, polarization and phagocytic activity. Transplanted cells exhibit long-term integration and photoreceptor rescue capacity. 3) hESC cultured on LN521 and LN111 exhibit efficient hepatocyte specification, maturation, function and stabilization of phenotype. The cells are highly organized and exhibit a significant increase in P450 metabolic enzyme functions. 4) LN-521 dramatically improves muscle cell proliferation and differentiation performance with more consistent and reliable differentiation over long-term culture.

T-2068

DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO TWO DISTINCT TYPES OF PANCREATIC PROGENITORS

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The generation of pancreatic β cells from human pluripotent stem cells (hPSCs) in vitro would provide a renewable source for cell therapy and disease modeling in diabetes. There are two types of pancreatic progenitors appearing during pancreatic development, which can be distinguished based on the expression of two transcription factors (TFs) (PDX1 and NKX6.1). Recently, it has been reported that the progenitors expressing both PDX1 and NKX6.1 can be differentiated into mono-hormonal pancreatic β cells (mature), while those expressing only PDX1 can only be differentiated into poly-hormonal β cells (immature). To generate fully functional pancreatic β cells, it is essential to obtain pancreatic progenitors expressing both TFs. Therefore; in this study our aim was to establish an efficient method to generate two distinct types of pancreatic progenitors from hPSCs, which can be used to understand molecular mechanisms underlying pancreatic β cell development. In this study, a four-stage protocol that efficiently converts hPSCs into pancreatic progenitors was used. A combination of several growth factors and small molecules were applied to generate different stages of pancreatic lineages. Our results showed that we were able to differentiate hPSCs into enriched population of definitive endoderm expressing specific endodermal markers SOX17 and FOXA2. By modifying culture condition, we obtained two different populations of pancreatic progenitors (PDX1+/NKX6.1+ and PDX1+/NKX6.1-). There were clear differences between hiPSCs and hESCs in their differentiation and maturation capabilities. Interestingly, pancreatic endocrine clusters were observed in hiPSC-derived NKX6.1+ progenitors, which were not seen in hESC-derived NKX6.1+ progenitors. These findings indicate that a selective differentiation protocol for pancreatic progenitors was established in our lab. This will improve our understanding of the pathways regulating the specification and maturation of pancreatic β cells in vitro. Also, it can be used to understand the pathophysiology of certain forms of diabetes.

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T-2070

RECONSTRUCTION OF PERIODONTAL ENVIRONMENT WITH BMP-6 AND THERMOSSENSITIVE HYDROGEL ENHANCES MOUSE PERIODONTAL REGENERATION FROM IPS

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Periodontal diseases that cause significant destruction of alveolar bone, periodontal ligament (PDL) and cementum may lead to progressive oral dysfunction. Periodontal tissue regeneration is the ultimate goal for periodontal disease treatment to reconstruct the structure and functions. However, due to the lack of proper microenvironment for transplanted cells, the regenerative efficiency was low. Here we applied an injectable and thermosensitive carboxymethyl-hexanoyl chitosan nanogel (CHC) to enhance stem cell delivery and engraftment. We combined CHC with BMP-6 (bone morphogenetic protein-6) and iPSCs to apply on rat periodontal defect, and found synergistic effects of iPSCs and BMP-6 on periodontal tissue regeneration with notable increase on bone and cementum formation. New connective tissue was observed in groups containing CHC but not without CHC. However, new PDL development was only found in group containing iPSCs, BMP-6 and CHC (iPSC-BMP-6-CHC). The 3D bio-scaffold composed by iPSC-BMP-6-CHC predominantly constructed the architecture of periodontal tissues within one week, and the mRNA levels of POSTN, OPN and CAP were significantly upregulated. An in vivo study revealed that CHC-encapsulated iPSCs with BMP-6 promoted mineralization as well as the formation of new connective tissue and PDL. Therefore, we suggest that CHC-encapsulated iPSCs with BMP-6 provides a new strategy to enhance periodontal regeneration.

T-2072

ACCELERATING AND SYNCHRONIZING THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED NEURAL STEM CELLS INTO NEURONS BY PREVENTING CELL PROLIFERATION

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Neural stem cells (NSCs) generated from human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs),

can be expanded in culture and further differentiated into neurons for disease modeling and chemical screening. Due to continuing proliferation of plated NSCs in culture in conventional neuronal differentiation medium, mixed cell populations including differentiated neurons and undifferentiated NSCs are often observed. Furthermore, cell aggregations are formed in culture and cell density increases along with the elongation of culture duration, which leads to the difficulty for interpreting end-point results. To solve these issues, we have developed a CultureOneTM supplement which can accelerate and synchronize the differentiation of hPSC derived NSCs into neurons by preventing the proliferation of undifferentiated NSCs in culture. With the treatment of CultureOneTM supplement, differentiated neurons are evenly distributed across the culture surface with extensive neurite networks and very little cell aggregation. Immonucytochemical staining showed that differentiated neurons expressed neuronal marker MAP2 or HuC&D with very few SOX1 positive undifferentiated NSCs at 2 weeks of differentiation. At 4-5 weeks of differentiation, the differentiated neurons expressed mature neuronal markers neurofilament and synaptophysin. To investigate the mechanism of CultureOneTM supplement actions, differentiating neurons were incubated with thymidine analog. By comparing with differentiating neurons without CultureOneTM supplement, the treatment with CultureOneTM supplement significantly decreased EdU positive cells without induction of cell death marker Caspase 9 expression, which suggests that the treatment with CultureOneTM supplement prevents cell proliferation. Upon depolarization with KCl, the signals of calcium influx of the differentiated neurons with the treatment of CultureOneTM supplement were much greater than untreated neurons, indicating the treatment with CultureOneTM supplement accelerates the maturation process of differentiating neurons. By using CultureOneTM supplement, the evenly distributed neurons with more maturity are more favorable to manual or automated imaging for quantification.

T-2074

INDUCTION OF MOUSE NEURAL CREST CELLS FROM INDUCED PLURIPOTENT STEM CELLS IN VITRO

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Neural crest cells (NCCs) are a group of embryonic cells which migrate long distances from the neural tube (i.e. developing central nervous system) to different locations of the embryos to form a multitude of derivatives. Based on their migration ability and multipotency, NCCs could be a potential cell source for cell therapy for birth defects involving abnormal NCC development such as Waardenburg syndrome and Hirschsprung's disease. However, the limited availability of NCCs directly from embryos or primary cultures is one of the main difficulties in developing this cell type into a useful cell source for cell therapy. With the discovery of induced pluripotent stem cells (iPSCs) which possess an unlimited ability of self-renewal, studies began to focus on the in vitro induction of NCCs from iPSCs. In the present study, we sought to develop a robust and efficient protocol to induce NCC formation from mouse iPSCs in vitro. We first induced mouse iPSCs to form crestospheres by a combination of growth factors FGF2, EGF and BMP4. Six days after induction on a non-adhesive surface, round and compact crestospheres derived from iPSCs were observed. Cells within the spheres strongly expressed NCC markers Sox10, AP-2a, p75, Slug, Snail, id2 and id3., and flow cytometric analyses showed that more than half of the cells were p75 immunoreactive. Next, cells were induced to emigrate from the crestospheres on a laminin-coated culture surface in a culture medium containing FGF2 and EGF. Emigrated cells were immunoreactive to NCC markers Sox10, Ap2a, nestin and FoxD3. The final step was to keep the induced cells proliferative and immunoreactive to the NCC markers. This was achieved by culturing them in a medium containing GDNF, in addition to other components. The induced NCCs were then assessed for their migration and differentiation by transplanting them to an embryonic gut explant culture ex vivo. Four days after transplantation, induced NCCs migrated long distances from the transplantation site and differentiated into TuJ1+ neuronal cells. Hence, our results demonstrated that with a three-step protocol involving the formation of crestospheres, iPSCs could be effectively induced into NCCs which were able to undergo extensive migration and differentiation into early neurons.

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T-2076

IDENTIFICATION OF ISOMIRS DURING CARDIAC DIFFERENTIATION: A POTENTIALLY SIGNIFICANT AMPLIFICATION SIGNAL OF THE MIRNOMA

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During pluripotent stem cell (PSC) differentiation significant changes in microRNAs expression have been reported. Deeper analyses of microRNA expression revealed that many of them are expressed with small, though potentially functional, changes in their sequences. These forms have been termed isoMIRs. In this study we describe isoMIR expression profile during cardiac differentiation from PSC. High-troughput sequencing of small RNAs (up to 50 bases) was made and analyzed. Three different cell populations were included: First, PSC grown in feeder free conditions. Second, mesoderm progenitor cells (MPC) obtain by a mesoderm specific differentiation protocol and CD326-/CD56+-based isolation. Third, isolated cardiomyocytes (CM) derived from PSC at 21 days of differentiation. Three replicates were performed for each group. Computational analysis was done under a mirDeep-based pipeline for the microRNAs and the Isomir-SEA tool and guidelines for isoMIRs identification. The latter corresponding to the 5'-end of the mature form of the microRNA (iso5), the 3'-end (iso3) and those microRNA with SNPs in their sequence. Approximately 700 hundred microRNA were found to be expressed in the three groups. We performed a fuzzy clustering and identified five general patters of microRNA differential expression, including fast-lowering, slow-lowering, up-and-down, fast-increasing and slow-increasing microRNAs. Further analysis of the microRNAs contained in this pattern showed a close relationship to microRNAs families and microRNAs genome clusters. The isoMIR analysis showed a widely disperse expression of the isoforms, particularly the iso3 (60±4.2% for PSC, 68.4±1.5% for MPC, and 61.3±5.6% for CM). Interestingly, the percentage of iso5 decreases significantly in the cardiac stage (7.2±0.8%, 7.8±0.7%, and 2.9±1%). Of note, changes in the 5'-end of the microRNAs are associated to modifications in the complementary seed, hence, broadening their specificity for target mRNAs. Finally, we could identify several iso5 isoMIRs corresponding to pluripotent-related microRNAs (i.e. the mir-302 family) that were significantly expressed. In conclusion, a wider analysis of the short non-coding RNAs during cardiac differentiation exposes an extended network of microRNAs and their respective isoMIRs.

PLURIPOTENT STEM CELL: DISEASE MODELING

T-2080

USING CHILD PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS TO MODEL ABCA3 DYSFUNCTION IN VITRO

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Childhood interstitial lung disease (chILD) can be caused by autosomal recessive mutations in ATP binding cassette member A3 (ABCA3), a lamellar body associated lipid transporter expressed in alveolar epithelial type II cells (AEC2s). Dysfunction of ABCA3 is thought to cause AEC2 injury by disrupting surfactant biogenesis, resulting in lung remodeling. AEC2s are difficult to study in cell culture due to their propensity to transdifferentiate, and inability to adequately proliferate. Using patient-specific induced pluripotent stem cells (iPSCs) as an inexhaustible source of AEC2s, we sought to engineer an in vitro model of ABCA3 deficiency. We reprogrammed blood and fibroblast samples of patients with homozygous E690K, W308R, and 806insGCT ABCA3 mutations spanning two major types of ABCA3 mutations. Using gene editing technology, we targeted a Tomato fluorochrome reporter to the Surfactant protein C (SFTPC) gene, the first locus specifically activated during the differentiation of distal lung epithelial progenitors. Using this reporter, we established a protocol which generated organoids containing putative Tomato+ cells that expressed AEC2-specific transcripts at levels similar to primary lung tissue controls by RT-qPCR and observed lamellar bodies by electron microscopy. Using this protocol, untargeted E690K iPSC were differentiated to organoids in 3D matrigel at which time they robustly expressed transcripts specific to AEC2s including—ABCA3, LPCAT, and SFTPC. Furthermore, we generated a SFTPC Tomato targeted E690K line which will enable us to identify, purity, and study AEC2-sepecific effects of ABCA3 deficiency. Future work will focus on the phenotyping and gene correction of AEC2s generated in all three ABCA3 deficient cell lines. We demonstrated the ability to generate putative AECs of sufficient maturity to potentially model chILD caused by mutations in ABCA3. Using this protocol, we will generate AEC2 models of different ABCA3 mutations compared to their gene-corrected controls. Upon complete characterization, these models will allow robust testing of novel drugs

and gene editing techniques for potential use in patients with ABCA3 mutations.

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T-2082

PROOF OF CONCEPT FOR CELL/GENE THERAPY OF HEMOPHILIA B WITH PATIENT'S SPECIFIC IPSC-DERIVED HEPATOCYTES AFTER GENETIC CORRECTION WITH CRISPR/CAS9 TECHNOLOGY

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Hemophilia B (HB) is a genetic disorder characterized by a reduced activity of circulating clotting factor IX (FIX), synthesized by hepatocytes. Current treatment based on regular intravenous injections of FIX is very restrictive, costly and only palliative. Gene therapy trials show promising results but their long-term efficacy is still unknown. It is therefore important to explore other treatment strategies. To demonstrate the feasibility of a gene/cell therapy approach using patient's iPSCs, we reprogrammed skin fibroblasts from a severe hemophilia B patient (FIX activity < 1%). One iPSC clone was deeply characterized by conventional techniques demonstrating self-renewal and pluripotency. Karyotype of the iPSCs was normal and DNA sequencing confirmed the presence of the missense patient's F9 mutation (E433K). We then targeted, using the CRISPR/Cas9 technology, the genomic insertion of a therapeutic cassette including the apolipoprotein AII (APOAII) promoter driving the expression of a F9 mini-gene bearing the Padua mutation to enhance FIX specific activity. One third of the amplified clones showed accurate monoallelic integration at the targeted AAVS1 safe harbor locus. The corrected or non-corrected iPSCs were differentiated into hepatocytes expressing specific markers like HNF4α and albumin. Due to the promoter used, the FIX mRNA expressed by the therapeutic cassette is detected during the early stages of differentiation whereas the RNA of the non-corrected clone is only detected at late stages. Under vitamin K treatment, both corrected and non-corrected differentiated hepatocytes produced FIX detectable both by immunostaining and western blotting. FIX activity measurements are currently performed and should confirm clotting activity in corrected cells. In parallel, the therapeutic efficacy of the correction in vivo is being assessed by transplantation of hepatocytes derived from corrected iPSCs into the liver of newborn HB mouse model.

T-2084

USING AN IPSC LINE WITH TRIPLICATION OF THE A-SYNUCLEIN LOCUS TO MODEL PARKINSON'S DISEASE FOR DRUG DISCOVERY

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Parkinson's Disease (PD) is a debilitating neurodegenerative disorder characterized by the loss of neurons in the central and peripheral nervous system (CNS and PNS). The causes for degeneration of neurons in PD are unknown, however clues have been provided through studying the underlying genetics of the disorder. SNCA, encoding alpha-synuclein (α-synuclein), was the first gene linked to PD, its duplication or triplication leads to an autosomal dominant form of familial PD, with an early onset and fast progression of the disease. SNCA protein aggregates are the main constituent of Lewy bodies, intracellular inclusions found in post-mortem PD brains, supporting a causative association of elevated neuronal α-synuclein levels and PD pathogenesis. The reduction of endogenous neuronal human α-synuclein levels thus represents a promising therapeutic approach. Previous approaches to lower the levels of α-synuclein within neurons were limited by their focus on modifying specific protein degradation pathways (e.g. proteasome, autophagy), a specific target (e.g. lamp2a), or a specific compound (e.g. trehalose). Furthermore, these activities often used in vitro cell models that did not reflect the complex phenotype of the human disease. Recent advances in human induced pluripotent stem cell (hiPSC) technology provide an opportunity to study aspects of PD in a human context. We therefore used neurons from PD-patient iPSCs carrying the SNCA locus triplication and an isogenic control with a single copy of the locus in order to identify novel compounds that would reduce endogenous α-synuclein levels without focusing on a predefined pathway. Here, we show data on the in vitro differentiation of these iPSC lines into neurons. We confirmed the previously reported two-fold increase of α-synuclein in neurons differentiated from the triplication line when compared to levels in neurons differentiated from the isogenic control. We are planning novel assays with these PD-iPSC derived neurons for drug discovery approaches.

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T-2086

ESTABLISHMENT OF A HIPSC-BASED NEURAL MODEL OF KLEEFSTRA SYNDROME ASSOCIATED WITH AUTISM

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Kleefstra syndrome (KS) is a rare genetic disorder that presents with a clinical phenotype including developmental delay, childhood hypotonia and may be associated with symptoms of autism spectrum disorder (ASD). Investigations using human induced pluripotent stem cell (hiPSC) technology to model autism-related syndromes with known genetic background have been described, yet, there has been no report on in vitro modeling of KS. The aim of the present study was to establish a patient-derived hiPSC-based in vitro disease model of KS accompanied by ASD (KS+ASD). HiPSCs were established from blood samples of a patient with KS+ASD (carrying a premature termination codon mutation in the euchromatic histone lysine methyltransferase 1, EHMT1 gene) and two healthy subjects. The hiPSCs showed ESC morphology, normal karyotype, expressed pluripotency markers, and were able to spontaneously differentiate into cells of the three germ layers. Determination of EHMT1 mRNA and protein expression demonstrated functional haploinsufficiency of the gene in the patient-derived cell cultures. The hiPSCs successfully differentiated into neurons as demonstrated by neuron specific immunolabeling for MAP2 and NF200, electronmicroscopic visualization of synapses and electrophysiological detection of ionic (sodium, potassium) currents and action potentials. Neurite morphology was significantly compromised on multiple endpoints, including full and maximal length of neurites, number of neurite roots and endings in the KS+ASD condition in comparison to controls. The number of neuritic protrusions was also reduced in the KS+ASD cultures compared to controls. Calcium currents evoked by glutamate did not differ between KS+ASD and controls, however, administration of acetyl-choline induced larger calcium currents in the KS+ASD cell cultures. Gene expression patterns of 180 ASD-associated candidate genes were investigated by qRT-PCR, showing significantly altered expression of ARX, SIX3, and HCN1 genes relative to both controls. The present iPSC-derived neuronal cultures represent an in vitro model system for KS which may serve to obtain a better understanding of the underlying pathophysiology of KS and potentially that of ASD.

T-2088

MOLECULAR ANALYSIS OF FMR1 REACTIVATING TREATMENTS FOR FRAGILE X SYNDROME IN VITRO AND IN VIVO

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Fragile X syndrome (FXS), the most common heritable form of intellectual impairment, is caused primarily by a CGG repeat expansion mutation in the FMR1 gene that triggers its epigenetic silencing. Restoration of FMR1 transcription by compounds that target repressive epigenetic marks was proposed as a therapeutic strategy for this disorder. We have previously shown that treatment of fragile X syndrome patient derived iPSCs and their neural derivatives with the DNA methyltransferase (DNMT) inhibitor 5-azacytidine is able to demethylate and reactivate the silenced FMR1 locus. In this study, we aimed to assess the ability of additional epigenetic modifiers to counteract the silencing of FMR1, and to analyze the efficacy of FMR1 reactivating treatments in the in vivo context. Using an immunostaining based imaging assay, we screened a collection of 140 epigenetic modulators for the ability to reactivate FMR1 expression in fragile X syndrome iPSCs. While nucleoside DNMT inhibitors induced the highest levels of FMR1 expression, several other compounds were able to potentiate the effect of demethylating treatment compared with DNMT inhibitors alone. Next, we aimed to establish an in vivo system for analysis of FMR1 reactivation. Systemic treatment with DNMT inhibitors of mice carrying transplants of differentiated FXS-iPSCs was able to robustly induce FMR1 expression in the affected cells. Treated transplants were analyzed in various time points following treatment withdrawal to assess the long term effects of FMR1 reactivation. Our system presents a novel platform for analysis of reactivating treatments for fragile X syndrome, which can help to assess the in vivo relevance of therapeutics identified in vitro.

T-2090

CHARACTERIZATION OF LESCH-NYHAN SYNDROME USING HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS

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Lesch-Nyhan syndrome (LNS) is a rare neurodevelopmental disorder caused by the deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) which plays an important role in the purine metabolism pathway. The symptoms of LNS are hyperuricemia, motor dysfunction, mental retardation and self-injury. Although hyperuricemia can be well managed by allopurinol treatment, currently there are no effective medications for neurological abnormalities. Since HPRT knockout mice do not show any behavioral phenotypes observed in LNS patients, alternative disease models are needed to reveal the mechanisms linking the HPRT deficiency to the neurological abnormalities. To this end, we have generated iPSC lines from LNS patient fibroblasts. We examined the HPRT gene expression levels among various cell types derived from human embryonic stem cells, and found that HPRT gene is strongly induced during the differentiation of dopaminergic neurons compared to other kinds of cells. It is reported that LNS patients have fewer dopaminergic neurons which might cause neurological symptoms, therefore we analyzed dopaminergic neurons induced from HPRT-deficient iPSCs. We found that the levels of purine metabolites are disturbed in HPRT-deficient dopaminergic neurons. Finally, we performed rescue experiments with human recombinant HPRT protein and demonstrated that the correction of purine metabolism could restore the phenotype of HPRT-deficient iPSCs. These results suggest that HPRT-deficient iPSCs are useful for disease modeling of LNS and could serve as a preclinical model for developing enzyme replacement therapy.

T-2092

METABOLIC PROFILING OF MOTOR NEURONS DERIVED FROM PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by progressive degeneration of upper and lower motor neurons of the spinal cord, brainstem, and motor cortex. Familial ALS can be caused by various mutations in SOD1, TDP43, FUS/TLS or C9ORF72 hexanucleotide repeat expansion. Being constantly active, neurons have very high energy demands. Dysregulation and mitochondrial dysfunction in motor neurons have been shown to contribute to ALS pathogenesis. Recent reports indicate that body physiology and energy metabolism in patients with ALS are impaired. However, the molecular profile of energy metabolism in motor neurons and its roles in ALS pathogenesis and progression remain poorly understood. In this study, we will be profiling metabolic regulation and mitochondrial function in ALS motor neurons derived from induced pluripotent stem cells (iPSCs). We performed gene expression profiling of metabolic enzymes and mitochondria genes in wild-type, SOD1L144F and TDP43G298S motor neurons, and found that ALS motor neurons show an over-active glycolytic pathway coupled with possible defects in mitochondrial biogenesis. This suggests that metabolic homeostasis imbalance may contribute to motor neuron death in ALS. We are currently performing in depth profiling of metabolic changes in ALS and WT motor neurons and future work involves reversing the pathogenic metabolic changes to achieve normalcy in ALS.

T-2094

GENERATION OF HUMAN NEONATAL DIABETES MELLITUS-INDUCED PLURIPOTENT STEM CELLS

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Diabetes mellitus (DM) results from destroys pancreatic beta cells by autoimmune attack or begins at older age with insulin resistance in target organs, beta cell dysfunction and deficiency. Neonatal DM is caused by beta cell dysfunction due to mutations in the genes encoding the ATP-sensitive potassium channel (KATP channel). However, molecular mechanisms how mutations in the KATP channel are associated with dysfunctions of pancreatic beta cells remain poorly understood. To explore cellular modeling of neonatal DM, induced pluripotent stem cells (iPSC) were generated from human dermal fibroblasts derived from a neonatal DM patient (ND-iPSCs). The patient has a mutation on KCNJ11 gene (c.602G>A) encoding Kir6.2 subunit of KATP channel complex. The neonatal DM-iPSCs normally expressed pluripotency-associated genes at the protein levels. To study whether neonatal DM phenotypes recapitulate in pancreatic developmental process in vitro, neonatal DM-iPSCs were differentiated into insulin-producing endocrine cells (ECs) by using stepwise protocols. Like wild type

(WT)-iPSCs, neonatal DM-iPSCs normally developed to definitive endoderm (DE), pancreatic endoderm (PE), endocrine progenitors (EPs) and endocrine cells (ECs). Differentiated cells derived from DM-iPSCs at respective developmental stages expressed developmental stage-specific markers, respectively. In particular, DM-ECs expressed endocrine hormones such as c-peptide, insulin, pancreatic polypeptide and somatostatin, like WT-ECs. These MD-iPSCs will be very useful for studying molecular and cellular mechanisms on pathophysiology of neonatal DM.

T-2096

DISEASE MODEL OF DIABETES USING CRISPR GENE-EDITING IPSC FOR DRUG SCREENING

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Human induced pluripotent stem cells (hiPSCs) represent a valuable system for the production of cell types with a clinical relevance for disease modeling and drug screening. Diabetes is a major global healthcare issue with huge negative social and economic consequences. There are many forms of diabetes including type 1 immuno-mediated diabetes, type 2 complex diabetes and multiple forms on monogenic diabetes MODY and neonatal forms. The authors have successfully generated modified hiPSC lines using CRISPR technology to integrate point mutations in both the HNF1-alpha and KCNJ11 genes. This has enabled the generation of exemplar MODY 3 and neonatal diabetes disease models respectively. An optimized platform using defined conditions was used to direct differentiate the gene-edited and wild-type lines into pancreatic diabetes disease models in vitro. In the first stage of iPS pancreatic cell generation, iPSCs are differentiated into definitive endoderm (DE) cells. The second stage differentiates the DE cells into anterior definitive endoderm (ADE) cells and in the third stage; ADE cells differentiate into pancreatic progenitors. During the fourth stage of the protocol, the progenitors mature to a point where a significant proportion of the cells express key pancreatic beta cell markers and exhibit glucose-responsive C-peptide production at physiologically relevant levels. Analysis of the iPSCs revealed that heterozygous and homozygous point mutations could be successfully introduced. Subsequent studies on the resultant pancreatic beta cell populations revealed that in the case of HNF1-alpha disease model cells gene expression levels of key sets of genes known to be regulated by HNF1-alpha were decreased as expected. Furthermore, the KCNJ11 model showed deregulated response to glucose stimulation in contrast with its wild type control. Both models are useful in elucidating the underlying mechanisms of these conditions and can also be used as a tool for drug screening. Future work is now focused on other monogenic and complex type 2 diabetes with studies ongoing to systematically analyze genome-wide association studies which have identified dozens of disease implicated loci (>44 genes), and systematically knock them out using CRISPR technology and therefore making several other disease models available.

T-2098

PATIENT-SPECIFIC DISEASE MODELING USING REPROGRAMMED VASCULAR SMOOTH MUSCLE CELLS

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Smooth muscle cells (SMC) display phenotypic plasticity, which play an important role in remodeling the vasculature during vascular disease progression. Therefore, the development of effective patient-specific therapeutic strategies for vascular diseases would greatly benefit from the understanding and modulation of SMC function. However, it is often difficult to isolate a large number of SMC through biopsy, and in vitro expansions are limited due to low proliferation and quick senescence acquisition of SMC in culture. In order to find an alternative autologous source for SMC, we have explored both iPSC reprogramming based differentiation and direct transdifferentiation to obtain functional SMC from the whole blood sample of donors. The resulting reprogrammed SMC were verified phenotypically and functionally using qRT-PCR, immunofluorescent staining, and calcium transient activity imaging. In addition, we studied these reprogrammed SMC in a physiologically relevant setting by fabricating tissue-engineered blood vessels (TEBV) using dense collagen gel. Using pulsatile perfusion system, we tested the engineered vessel function in response to various vasoactive drugs. As such, our reprogrammed SMC that are functional and highly obtainable in large quantity opens a new venue for patient-specific vascular disease modeling.

T-2100

ELEVATED LEVELS OF ROS AND ANTIOXIDANT GENE EXPRESSION IN INNER EAR HAIR-LIKE CELLS DIFFERENTIATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS WITH MITOCHONDRIAL DNA MUTATION

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Mitochondria play an important role in the generation of energy of eukaryotic cells. Mutation in mitochondrial DNA (mtDNA) will cause mitochondrial disease. Myoclonus epilepsy associated with ragged-red fibers (MERRF) is one of mitochondrial diseases characterized by myoclonus, generalized epilepsy, ataxia and hearing loss. MERRF is primary due to an A-to-G transition at mtDNA 8344 (A8344G) that affects the gene encoding tRNALys whitch is important in oxidative phosphorylation (OXPHOS). The genetic mutation causes mitochondrial dysfunction will generate the reactive oxygen species (ROS) within mammalian cells. Whether the generation of ROS within inner ear mechanosensory hair cells implicated in hearing disorders is still unclear. In our study, we used human induced pluripotent stem cells (hiPSCs) with mtDNA A8344G mutation from patients with MERRF syndrome. When compared with control iPSC, MERRF-hiPSCs exhibited increased ROS production and fragmented mitochondrial morphology. We are therefore interested to investigate the induction capacity and mitochondrial function of hair cell-like cells (MERRF-HCs) differentiated from MERRF-hiPSCs. From our results, the ROS and antioxidant gene (such as catalase) expression levels in MERRF-iPSC-derived HCs were higher than control iPSC-derived HCs. Furthermore, these differentiated HCs also contained fragmented mitochondria. Our findings will help to develop the therapeutic strategies for hearing loss in mitochondrial diseases.

T-2102

CHARACTERIZATION & ROLE OF NEUREXIN1 (NRXN1) IN EARLY BRAIN DEVELOPMENT LINKED TO AUTISM SPECTRUM DISORDERS

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by impaired social communication and interactions by restricted and repetitive behaviors. Deletion in the Neurexin 1 (NRXN1) gene is associated with increased risk of ASD, a presynaptic transmembrane protein that bridge between pre-and postsynaptic neurons. However, the molecular mechanism by which NRXN1 deletion impacts ASD remains unclear. To elucidate the molecular consequences of NRXN1 deletion in early neural development, we carried out single cell RNA-seq analysis to investigate gene expression in early development of human neuroepithelial cells (NES) and differentiating neurons derived from iPSCs of patient and healthy individuals. Distinct clusters of interest and differentially expressed genes were identified by using Illumina system analysis. Number of genes (identified in this study) that undergo drastic changes in expression during the transition from NES to neuronal differentiation are referred to as candidate genes of ASD. These genes involved in synaptic formation/remodeling/maintenance; neurotransmission; chromatin modification and regulation of transcription factors. Further, we detected decreased gene expression of NRXN1 and increased expression of early and late astrocytes markers (S100Bα/β, GFAP) in patient-derived cells during differentiation whereas neuronal markers DCX and MAP2 expressions remained unchanged both in patient and healthy neurons. These findings are correlated with observations recorded from in vitro cells culture analysis. In short, these results strongly suggest that NRXN1 deletion: seems to affect balance between the glial-neuronal differentiation pathway in early brain development; likely to be responsible for the patient's impaired cognitive functions; and possibly involve in the pathophysiology of autism - offering the potential for new perspective on the understanding of this neurodevelopmental disorder.

T-2104

CHARACTERIZATION OF SKELETAL MUSCLE DIFFERENTIATED FROM HUMAN PLURIPOTENT STEM CELLS

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Skeletal muscle (SkM) comprises 40-50% of the human body mass and serves vastly important functions such as the ability to provide locomotion, respiration and metabolic control amoung various others. There are many diseases associated with muscle loss which can be caused by normal aging (sarcopenia), cancer (cachexia) and genetic mutations (muscular dystrophies). Treatment options for muscle diseases are very limited and the loss of normal muscle function affects patient's quality of life, independence and for some diseases their lifespan, thereby presenting a significant burden

to society and healthcare systems. Human pluripotent stem cells (hPSC) differentiated to SkM enables the development of muscle disease models as a tool for drug discovery or cell therapeutic approaches. Genea Biocells developed and published a robust, scalable and efficient protocol for producing SkM from hPSC. A common general limitation of human stem cell models is the still embryonic nature of the resulting cells which was also observed for hPSC-derived SkM. In more recent research we conducted high-throughput screens to address this shortcoming and to make more mature, adult-like SkM. This has to the discovery of compounds that improve MyoD positive cell formation and myoblast fusion to multinucleated myotubes. The resultant muscle cells are morphologically similar to that of primary myoblast cultures. The maturity and contractile ability of hPSC-derived skeletal myotubes produced using this improved method was assessed using readouts including action potentials (multi-electrode arrays, Calcium imaging), spontaneous contraction and pharmacological regulation of contraction. These parameters will be useful measures to study muscle related diseases, to identify disease phenotypes and to evaluate the mechanism of action and efficacy of drug leads.

Funding Source: California Institute for Regenerative Medicine (CIRM)

T-2106

GENERATION OF A PATIENT-DERIVED DOPAMINERGIC CELL MODEL OF AROMATIC L-AMINO-ACID DECARBOXYLASE (AADC) DEFICIENCY

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Aromatic L-Amino Acid Decarboxylase (AADC) deficiency is a severe pharmacoresistant neurological disorder due to inherited autosomal recessive mutations in the DDC gene. The resultant loss in AADC enzyme activity impacts on monoamine synthesis, leading to reduced levels of dopamine and serotonin. Affected patients present with marked neurodevelopmental delay, hypotonia, oculogyric crises and autonomic dysfunction. We have generated induced pluripotent stem cell (iPSC) lines from skin fibroblasts derived from two patients with AADC deficiency and one age-matched control subject. One patient harboured a homozygous missense mutation (p.R347Q) and the other was a compound heterozygote for a nonsense variant (c.C102T) and missense mutation (p.L408I) in DDC. Generated iPSC lines were confirmed as being truly pluripotent, then successfully differentiated into midbrain dopaminergic (mDA) neurons, with characteristic neuronal morphology and expressing tyrosine hydroxylase (TH) and microtubule-associated protein 2 (MAP2). Using high performance liquid chromatography (HPLC) techniques, we detected significantly lower AADC enzyme activity in patient mDA when compared to the age-matched control (P=0.0071). In conclusion, our iPSC-derived mDA neuronal model represents an ideal platform to further elucidate disease mechanisms, as well as to screen novel pharmacological agents to treat AADC deficiency.

Funding Source: AADC Research Trust; UCL IMPACT scholarship

T-2108

REPROGRAMMING OF GASTRIC CANCER CELLS INHIBITS ONCOGENIC FUNCTION OF HOXA13-LONG NONCODING RNA HOTTIP PATHWAY

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Reprogramming of cancer cells into induced pluripotent stem cells (iPSCs) is a compelling idea for inhibiting oncogenesis, especially the role of homeobox proteins on stemness and cancer features. We examined the role of various long noncoding RNAs (lncRNAs)-homeobox protein HoxA13 axis on the switching of the oncogenic function of bone morphogenetic protein 7 (BMP7), which is significantly lost in the gastric cancer cell derived iPSC-like cells (iPSLCs). BMP promoter activation occurred through the corecruitment of HoxA13, mixed-lineage leukemia 1 lysine N-methyltransferase (MLL1), WD repeat-containing protein 5, and lncRNA HoxA transcript at the distal tip (HOTTIP) to commit the epigenetic changes to the trimethylation of lysine 4 on histone H3 in cancer cells. By contrast, HoxA13 inhibited BMP7 expression in iPSLCs via the corecruitment of HoxA13, Enhancer of zeste homolog 2, Jumonji and AT rich interactive domain 2, and lncRNA HoxA transcript antisense RNA (HOTAIR) to various cis-element of the BMP7 promoter. Knockdown experiments demonstrated that HOTTIP contributed positively, but HOTAIR regulated negatively to HoxA13-mediated BMP7 expression in cancer cells and iPSLCs, respectively. These findings indicate that the recruitment of HoxA13–HOTTIP and HoxA13–HOTAIR to different sites in the BMP7 promoter is critical for the oncogenic fate of human gastric cells. Reprogramming

with OCT4 and JDP2 can inhibit tumorigenesis by switching off BMP7.

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T-2110

MULTIPARAMETRIC PHENOTYPIC ASSAYS FOR SCREENING COMPOUNDS IN NEURONS DERIVED FROM SPASTIC PARAPLEGIA TYPE 4 PATIENTS

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The use of iPSC models for drug discovery critically depends on the availability of robust differentiation paradigms and phenotypic assays that enable a fast read-out within hours or days after cell plating. We addressed these challenges in the context of spastic paraplegia type 4 (SPG4), the most frequent, autosomal dominant subtype of hereditary spastic paraplegia (HSP). We first established a standardized differentiation protocol yielding highly enriched cortical cultures comprising >80% glutamatergic neurons expressing the layer V/VI markers CTIP2 and TBR1. Cortical neuronal progenitors and neurons generated with this protocol can be cryopreserved as ready-to-use batches for downstream assays. In a second step, we developed three early phenotypic assays that could be transferred to a semi-automated 96-well drug testing setup for the readout of i) axonal swellings, a hallmark of HSP pathology that can already be reliably detected after 5 days in culture, ii) neurite outgrowth, which is reduced by 50% in SPG4 neurons already 24 hours after plating, and iii) growth cone area, which is increased 2.5 times compared to controls 24 hours after plating. We next applied candidate small molecules to this setup and identified five compounds that positively modulated ≤2 phenotypes; one candidate drug rescued all three phenotypes in SPG4 neurons without affecting healthy control neurons. We expect this multiparametric and rapid phenotyping approach to accelerate the study of pathomechanisms underlying HSP as well as the identification and development of drugs for therapeutic intervention.

T-2112

INDUCED PLURIPOTENT STEM CELLS-DERIVED HEPATOCYTES: DISEASE MODELLING AND CRISPR/CAS9-MEDIATED GENETIC CORRECTION FOR FAMILIAL HYPERCHOLESTEROLEMIA TYPE IIA

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Familial hypercholesterolemia type IIa (FH) is a liver genetic disorder caused by mutations in the low-density lipoprotein receptor (LDLR). As the cholesterol removal from the circulation is impaired, patients die from early cardiovascular diseases. Hepatocytes are the only cells able to metabolize cholesterol into bile acids and therefore, are the target for FH cell/gene therapy approaches. Using a non-integrative method, we have generated FH-induced pluripotent stem cells (FH-iPSCs) from a homozygous patient with an early stop codon in the LDLR sequence and demonstrated that they display the disease phenotype (absence of LDLR expression and functionality). The role of LDLR in VSV-G pseudotyped lentiviral vector entry, previously reported in the literature, was confirmed on these cells. Following a stepwise protocol mimicking liver embryogenesis, FH-iPSCs were differentiated into hepatocytes (iHeps) expressing transcription factors (HNF4α, HNF3β), specific proteins (albumin, α-1 antitrypsin), polarity markers (ZO-1) and Hepatitis C Virus (HCV) receptors (claudin1, CD81, SRB1). Treatment of these iHeps with Ataluren, a small-molecule currently tested for stop codon read-through in cystic fibrosis therapy, induced internalization of a fluorescent ligand of the receptor (Dil-LDL), suggesting a LDLR read-through translation. Then, FH-iPSCs were genetically corrected using the CRISPR/Cas9 technology targeting the AAVS1 safe harbor locus. A template plasmid containing the normal LDLR cDNA controlled by hepatic specific Apolipoprotein A2 promoter flanked by homologous recombination arms of the AAVS1 locus was constructed then electroporated into these cells. Accurate monoallelic homologous recombination has occurred in half (15) of the isolated clones and one of them was next differentiated into iHeps. In these cells, LDLR expression and functionality were restored as demonstrated by Western Blot and Dil-LDL internalization. The level of internalization corresponded to 62% of control iHeps derived from healthy donor iPSCs, in agreement with a monoallelic integration of the therapeutic cassette. Both corrected and non-corrected FH-iPSCs will now be used to study

the regulation of cholesterol pathways and to further understand the still debated role of LDLR in HCV entry.

REPROGRAMMING

T-2116

INVESTIGATING THE FUNCTIONAL MATURATION OF HUMAN INDUCED NEURAL PRECURSOR-DERIVED NEURONS

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A major limitation to studying and treating neurological disorders is a lack of access to live human neurons. Reprogramming somatic cells to induced pluripotent stem cells (iPSCs) is one strategy that has received a lot of attention for the generation of mature human neurons. Direct reprogramming technologies can also provide an alternative method to generate live human neurons. Our laboratory has developed the ability to directly reprogram adult human dermal fibroblasts (HDFs) into induced neural precursor cells (iNPs) by non-viral delivery of the neural genes SOX2 and PAX6. We can subsequently differentiate directly reprogrammed iNPs into human neurons exhibiting a variety of region-specific phenotypes including glutamatergic and GABAergic fates. To extend this work, the current study investigates the functional maturation of directly reprogrammed human iNP-derived neurons. HDFs were reprogrammed to iNPs by delivery of chemically-modified SOX2 and PAX6 mRNA in combination with a defined reprogramming medium. The resultant iNPs were then differentiated to a mixed population of neurons and astrocytes for a minimum of 30 days. Using quantitative TaqMan® PCR, we observed that expression of the pro-neural gene ASCL1, the ventral forebrain gene DLX2, the ventral telencephalic genes CTIP2 and MEIS2, the early glutamatergic gene TBR2, the glutamatergic gene VGLUT1 and the GABAergic gene GAD67 were upregulated during differentiation. The pre-synaptic genes SYP and SNAP25 were highly expressed throughout differentiation, as were the post-synaptic genes PSD95, SHANK2 and SAP97. Abundant levels of mRNA were also detected during differentiation for the voltage gated calcium and sodium channel genes, CACNA1C and SCN8A. However, iNP-derived neurons were not electrophysiologically functional after 30 days of differentiation. Human iNPs differentiated in astrocyte conditioned media developed more complex neuronal morphologies. Additional studies are underway to optimise the functional maturation of our iNP-derived neurons which will be confirmed by electrophysiological recordings and calcium imaging. Future studies will examine the functional integration of directly reprogrammed human iNPs on an established rat organotypic slice culture platform.

T-2118

FASTING-MIMICKING DIET INDUCES PANCREATIC LINEAGE REPROGRAMMING TO PROMOTE NGN3- DRIVEN B-CELL REGENERATION

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Stem cell-based therapies can potentially reverse organ dysfunction and diseases but the removal of impaired tissue and reactivation of the program leading to organ regeneration pose major challenges. Here, we show that a four-day fasting mimicking diet (FMD) decreases the levels of pancreatic β cells by 35%; an effect reversed by β-cell regeneration after re-feeding. In mice, this β-cell reconstitution follows a step-wise activation of Sox17 and Pdx-1 resembling that associated with pancreatic β-cell development, leading to Ngn3-driven generation of insulin-producing β-cells. FMD cycles restore insulin secretion and glucose homeostasis in both a type 2 (Lepr db/db) and a type 1 streptozotocin (STZ)-induced diabetes mouse models. In human pancreatic islets, fasting mimicking conditions promote similar changes in Sox2 and Ngn3 expression. In cells from T1D subjects, expression of these genes can be recapitulated by inhibition of downstream targets of IGF-1 (i.e. PKA and mTOR). This study provides the first example of a diet-induced lineage reprogramming and fate conversion in the adult pancreas, with potential therapeutic applications.

T-2120

INDUCED PLURIPOTENT STEM CELLS FROM A MARSUPIAL, THE TASMANIAN DEVIL (SARCOPHILUS HARRISII): INSIGHT INTO THE EVOLUTION OF MAMMALIAN PLURIPOTENCY

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The study of marsupial biology has both intrinsic and comparative significance, and may serve to stem the further loss of marsupial diversity due to extinction of species. Induced pluripotent stem cells (iPSCs) are adult somatic cells that have been re-programmed to

an embryonic stem cell-like state, providing a powerful research tool. We demonstrate the generation of Tasmanian devil (Sarcophilus harrisii) iPSCs (DeviPSCs), the first marsupial stem cells, from dermal fibroblasts by lentiviral transduction of human transcription factors: OCT4, SOX2, KLF4, NANOG, c-MYC and LIN-28. DeviPSCs display characteristic pluripotent stem cell colony morphology, with individual cells having a high nuclear to cytoplasmic ratio and alkaline phosphatase (AP) activity. DeviPSCs are leukemia inhibitory factor (LIF) dependent and have reactivated endogenous POU5F1 (OCT4), POU5F3 (POU2), SOX2, NANOG and DAX1 genes, retained a normal karyotype, and concurrently silenced exogenous human transgenes. Notably, expression of both POU5F1 and POU5F3 appears to reflect a naive state of pluripotency since both factors are known to be expressed by cells from the epiblast of marsupial pre-implantation embryos. Under culture conditions favouring differentiation, DeviPSCs readily formed embryoid bodies (EBs) and in vitro teratomas containing derivatives of all three embryonic germ layers. To date, DeviPSCs have been stably maintained for more than 45 passages. Thus, our DeviPSCs are important in understanding marsupial biology and the evolution of mammalian pluripotency. Significantly, these DeviPSCs will be an indispensable asset for stem cell-based regenerative and anti-cancer therapies against Devil Facial Tumour Disease (DFTD) which has threatened Tasmanian devils with extinction.

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T-2122

CONTEXT-DEPENDENT FUNCTIONS OF NANOG PHOSPHORYLATION IN PLURIPOTENCY AND REPROGRAMMING

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Nanog is a core pluripotency transcription factor that is critical for embryonic stem cell (ESC) self-renewal and the faithful reprogramming of somatic cells to induced pluripotent stem cells (iPSCs). Although Nanog is phosphorylated at multiple residues, the role of Nanog phosphorylation in ESC self-renewal is incompletely understood, and no information exists regarding its functions during reprogramming. Here we report our findings that Nanog phosphorylation is beneficial, although nonessential, for ESC self-renewal, and that loss of phosphorylation enhances Nanog activity in reprogramming. Mutation of serine 65 in Nanog to alanine (S65A) alone had the most significant impact on increasing Nanog reprogramming capacity. Mechanistically, we find that pluripotency regulators (Esrrb, Oct4, Sall4, Dax1, and Tet1) are transcriptionally primed and preferentially associated with Nanog S65A at the protein level due to presumed structural alterations in the N-terminal domain of Nanog. These results demonstrate that a single phosphorylation site serves as a critical interface for controlling context-dependent Nanog functions in pluripotency and reprogramming.

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T-2124

MARVELD1 IMPEDES MET PROCESS DURING MOUSE FIBROBLAST REPROGRAMMING

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Epithelial–mesenchymal transition (EMT) plays important roles in individual development and cancer progression. During the induction of iPSCs, a process that fibroblasts be reset into an embryonic stem cell like state via exogenous transcription factors, one of the initial changes is the fibroblasts transform into round cells and grow like tightly packed clusters. This process is known as MET, the opposite of EMT. Cancer related genes are also implicated in cell reprogramming including Yamanaka factors (Oct4, Klf4, Sox2 and c-Myc), P53, c-Jun and so on. Nuclear factor MARVELD1 (MARVEL domain-containing 1) is a novel tumor suppressor candidate, which widely expressed in human normal tissues but down regulated in lung carcinomas by promoter methylation. Remarkably, MARVELD1 overexpression reduced cell migration and increased cell-extracellular matrix adhesion in cancer cell lines. Our previous study found that the depletion of MARVELD1 triggered placenta accreta via promote trophoblast cell EMT process in mice. However, it remains unknown whether MARVELD1 play roles during somatic cell reprogramming, and, if so, what is the underlying molecular mechanisms. To answer these questions, we identified that MARVELD1 expression level was much higher in mouse fibroblasts than that of mouse embryonic stem cells. Accordingly, the expression of MARVELD1 was increased during the differentiation of mouse embryonic stem cells. More importantly, the knockout of MARVELD1 enhanced the efficiency of mesenchymal-epithelial transition during mouse somatic cell reprogramming, and the overexpression of MARVELD1 decreased the efficiency of mouse iPSCs generation. These data suggest that

MARVELD1 implicates in the reprogramming of mouse fibroblasts. Consequently, MARVELD1 decreases the efficiency of iPSCs generation through suppressing its initial MET process. As MARVELD1 suppresses both EMT and MET processes in mice, and it is mainly distributed in chromatin-enriched fractions, these data indicate that MARVELD1 performs its role in chromatin level, the underlying mechanisms are remain under investigated. This project helps us better understanding the molecular mechanisms of MARVELD1-mediated EMT-MET during stem cell differentiation and somatic cell reprogramming.

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T-2126

ROLES OF SMN IN SOMATIC CELL REPROGRAMMING AND NEURONAL DIFFERENTIATION IN THE MOUSE SMA MODEL

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Survival motor neuron (SMN) is an essential protein that plays important roles in the assembly of small nuclear ribonucleoproteins (snRNPs), the components of the RNA spliceosome. Deficiency of the ubiquitous SMN protein causes spinal muscular atrophy (SMA), the most common human neuron degenerative disease. U12-dependent minor spliceosome is preferentially affected in SMA cells, therefore leads to massive splicing failure and motor neuron apoptosis. In addition to the roles in neuron cells, we and others showed that SMN regulates stem cell pluripotency, division, proliferation and differentiation in Drosophila and mice. SMN is abundant in mouse oocytes and embryonic stem cells, but low in mouse embryonic fibroblast cells (MEFs) and neurons. Here we hypothesize that SMN plays important role during the reprogramming process of induced pluripotent stem cell (iPSC) formation. The objective of this study is to examine the roles of SMN in cellular reprogramming and neuronal differentiation in normal and SMA cells. SMN expression increased during the induced pluripotency reprogramming process by OKSM (Oct4, Klf4, Sox2 and cMyc) factors when C57BL/6 wild type (WT) mouse MEFs were used, along with the typical pluripotency markers including Nanog, Oct4 and SSEA1. Overexpression of SMN in WT cells improved the iPSC formation efficiency as judged by alkaline phosphatase (AP) activity; whereas knocking-down of SMN completely abolished the iPSC formation. Consistently, iPSC formation efficiency using SMA mouse tail-tip fibroblasts was extremely low. Importantly, overexpression of SMN in SMA tail-tip fibroblasts improved the iPSC derivation as well as the neurite in vitro differentiation, and teratoma formation. These findings indicated that SMN plays important roles during cellular reprogramming.

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T-2128

SYNCHRONOUS REPROGRAMMING AND GENE CORRECTION OF HUMAN HUTCHINSON-GILFORD PROGERIA SYNDROME DERMAL FIBROBLASTS

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Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare genetic disease characterized by accelerated aging in children. It is caused by a silent mutation in lamin A (LMNA) gene (c.1824 C>T) giving rise to a cryptic splice site, resulting in the production of an abnormally spliced mature mRNA, that on translation produces a truncated protein called Progerin which is 50 amino acids short. Progerin accumulates in a dominant-negative fashion, causing nuclear instability and loss of cellular proliferation, ultimately leading to an aging phenotype at an early age with high risk of cardiovascular disease and stroke. HGPS patient fibroblasts have previously been reprogrammed to generate induce pluripotent stem cells (iPSCs) for their use in cell therapy using integration based retroviral and lentiviral methods. These HGPS-iPSCs & HGPS fibroblasts have also been subject to gene editing using adenoviral vectors, which is arduous and time-intensive leaving genomic scars after selection process. In an effort to establish a streamlined autologous cell-based therapy for HGPS patients, we are developing a method for, in vitro, synchronous reprogramming and gene correction (SGRC) of HGPS patient fibroblasts via a single nucleofection event using episomal reprogramming factors (Oct4, Sox2, Klf4, l-Myc, Lin28, p53shRNA, miRNA 302/367 cluster) and Cas9 nickases with single-stranded oligodeoxynucleotide (ssODN) donor template to correct the LMNA mutation via homology-directed repair. We have successfully reprogrammed BJ and HGPS fibroblasts using the episomal factors via nucleofection. The use of a non-integrating reprogramming method along with convenient CRISPR technology for SRGC will eventually facilitate production of scar-free iPSCs in a timesaving manner, which can be used for downstream application like expansion and directed differentiation into vascular/

cardiac or other cell types to develop regenerative therapies for HGPS patients.

Funding Source: This research was supported by NIH grant R25-AG047843-01, which supports Howard University's Advancing Diversity in Aging Research (HUADAR) program, in which Dr. Antonei Csoka is Co-Investigator.

T-2130

SIRT1 AND SIRT2 DISTINCTLY REGULATE METABOLIC REPROGRAMMING DURING HUMAN INDUCED PLURIPOTENCY

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A hallmark of cancer cells is the metabolic switch from oxidative phosphorylation (OXPHOS) to glycolysis, a phenomenon referred to as the "Warburg effect", which is also observed in various stem cells such as human pluripotent stem cells (hPSCs) including human embryonic stem cells and induced pluripotent stem cells. Although this metabolic reprogramming appears to be a fundamental aspect of human induced pluripotency and stem cell fate control, the molecular mechanisms underlying the link between energy metabolism and stem cell fate are poorly understood. Here, we found that protein acetylation is a fundamental aspect of stem cell fate control and critically regulate induced pluripotency and metabolic reprogramming. In particular, we report that upregulation of SIRT1 and downregulation of SIRT2 is a molecular signature of hPSCs and that SIRT1 and SIRT2 play critical roles during human induced pluripotency. In addition, we found that altered SIRT1 and SIRT2 expression distinctly regulate energy metabolism, altering stem cell fate and functions such as reprogramming, pluripotent differentiation properties, self-renewal capacity, and survival. We will discuss our findings regarding the distinct roles of SIRT1 and SIRT2 as acetylation modulators in metabolic reprogramming during human induced pluripotency and stem cell fate control.

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T-2132

A PROTOCOL SUITABLE FOR THE GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM PERIPHERAL BLOOD CELLS FOR CLINICAL APPLICATIONS

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Induced pluripotent stem cells (iPSCs) generated from the individuals homozygous for HLA haplotypes have potential applications in regenerative medicine. Therefore, for the generation of immune matched clinical grade iPSCs, a project has been initiated in India for the banking of iPSCs from the donors with the most common HLA haplotypes. A major challenge in the clinical application of iPSCs is the establishment of a suitable protocol to generate clinical-grade iPSCs. We standardized an efficient and a cost-effective protocol to generate iPSCs from the cultured erythroid cells derived from peripheral blood mononuclear cells (PBMNCs). PBMNCs from normal donors were cultured in complete Stempro-34 serum-free medium in the presence of the cytokines, SCF, IL-3, EPO and IGF-1, for 8 days, which yielded >85% CD71+/CD235+ erythroid cells. These cells were transfected with oriP/EBNA-1-based episomal plasmids expressing OCT4, SOX2, KLF4, L-MYC, LIN28 and a p53 shRNA by nucleofection, and then they were plated on recombinant human Laminin coated plates. The cells were maintained for 6 days in Stempro-34 medium containing cytokines. Subsequently, the cells were cultured in Essential-8 medium containing b-FGF, with the medium change every day. After 15-20 days, iPSC colonies with the morphology of human embryonic stem cell colonies were isolated and expanded on either Vitronectin or Laminin coated plates. After 10 passages, these clones were characterized by immunofluorescence and real-time PCR analysis of pluripotency markers, and by hPSC ScorecardTM for the accurate prediction of pluripotency and trilineage differentiation efficiency based on a panel of 94 genes. These clones were cryopreserved by using E8 medium supplemented with 10% DMSO, and we could obtain excellent recovery of the iPSC clones after revival, with no obvious cell death after thawing and plating. We used this protocol for the generation of 27 highly pluripotent iPSC clones from three normal donors. The safety of the iPSC clones were also evaluated for the absence of endotoxins and mycoplasma during their expansion in cGMP compliant facility. Hence, this protocol will be used for the establishment of iPSC banks from the individuals homozygous for HLA haplotypes in India.

Funding Source: Department of Biotechnology, Ministry of Science & Technology, Government of India

TECHNOLOGIES FOR STEM CELL RESEARCH

T-2134

UTILIZATION OF A PHYSIOLOGICALLY RELEVANT HIPSC-CARDIOMYOCYTES FOR A CARDIOTOXICTY SCREEN OF COMPOUNDS WITH KNOWN MECHANISM OF ACTION

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hiPSC-derived cardiomyocytes (hiPSC-CMs) have emerged as a powerful system for the study of safety and toxicity profiles of therapeutic compounds under development. Cardiac toxicity plays an important role in the failure of therapeutic agents in late stages of clinical trials, as well as for the removal of approved drugs from the market. The Comprehensive in vitro Proarrhythmia Assay (CiPA) constitutes a novel safety screening proposal intended to replace current regulatory strategies that have failed to predict the acute cardiotoxic effects of developing drugs. Through the CiPA initiative, researchers from diverse organizations such as the FDA, academic institutions, and pharmaceutical companies are evaluating hiPSC-CMs as an integral tool for the safety assessment of novel therapeutic compounds. Key challenges under consideration for this elegant cellular system are sub-ideal cardiomyocyte geometry, sub-cellular structural organization and overall electro physiological maturity. We interrogated hiPSC-Cardiomyocytes contained in a pre-formatted physiologically-relevant platform to asses if cardiomyocyte alignment, translating into more physiological cellular geometry, enhanced expression of key cardiac physiology genes and improved calcium handling would translate into better sensitivity, specificity and predictivity of the effects of compounds included in the pro-arrhythmia assay. Here we describe the behavior of this novel hiPSC-CM platform challenged with those compounds in a high throughput calcium flux assay. Further, we contrast those results with data gathered in multi-electrode array assays.

T-2136

EXPANSION OF CD34+/CD90+/CXCR4+ WITH CD146 + WHARTON'S JELLY MESENCHYMAL STROMAL CELLS

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The use of umbilical cord blood (UCB) for hematopoietic stem cell (HSC) transplantation is limited by the amount of CD34 + cells present in the units (UCB). The objective of this work was to determine the expansion factor of CD34+/CD90+/CXCR4+ of UCB obtained by static culture for 12 days. The UCB were cryo-preserved, thawed and the enriched CD34+ population was obtained, which were co-cultured with a subpopulation selected from CD146+ Wharton's jelly mesenchymal stromal cells and encapsulated in 2% calcium alginate. Culture conditions were RPMI-1640 medium supplemented with 10% SFB and human recombinant cytokines 5ng/ml TPO, 5 ng/ml SCF, 5 ng/ml FLT3-1, 2 ng/ml IL 3 and 1ng/ml DLK-1, the ratio of mesenchymal stem cells and hematopoietic stem cells is 2:1. The expansion factor of CD34+/CD90+/CXCR4+ was (12-day) 4,64 fold increase. This model suggest the feasibility of expansion of cryopreserved UCBs and increase CD34+/CD90+/CXCR4+ primitive populations using cytokines non-saturating concentrations and CD146+ Wharton's jelly mesenchymal stromal cells.

Funding Source: The research project was funded by the COLCIENCIAS y CryoHoldco/ Stem Regenerative Medicine.

T-2138

A NOVEL BIOCOMPATIBLE FLUORESCENT NANOPARTICLE ENABLES ENHANCED LIVE CELL TAGGING AND TRACKING OF MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) are adult stem cells which have the capacity for multi-lineage differentiation, giving rise to a variety of mesenchymal phenotypes such as osteoblasts (bone), adipocytes (fat), and chondrocytes (cartilage). Recently, interest in understanding the biology of MSCs has increased, especially for their therapeutic potential for a variety of diseases. Long-term noninvasive cell tracking

by fluorescent probes is of great importance to life science and biomedical engineering including stem cell researchers. Current methods used to fluorescently tag stem cells have been limited by short signal duration, high background auto-fluorescence or lengthy cell line generation using GFP. We have developed a biocompatible fluorescent nanoparticle which relies on Aggregation Induced Emission (AIE) technology that is highly resistant to fluorescent signal quenching. These particles enable highly efficient live cell fluorescent tagging while retaining fluorescent signal for up to 10 days in vitro and 41 days in vivo using both cancer and mesenchymal stem cell lines. These nanoparticles will open new avenues in the development of fluorescent probes for following biological processes in stem cell biology applications.

T-2140

A LIVE FLUORESCENT PROBE FOR DETECTING STEM CELL SENESCENCE

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Mesenchymal stromal cell (MSC) therapy represents a growing therapeutic and commercial value proposition. Given the need for out-scaling MSC expansion to generate high cell doses required for therapy, monitoring and quantifying the onset of cellular senescence during scale-up assumes significance. Existing methods for measuring cellular senescence suffer from poor alignment with scalar bioprocesses, due to the need for cell fixation or chemical treatment, dependence on pH, and fail to provide for real time, in-process measurements. To this end, we performed high-throughput screening of a compound library, consisting of cell-permeable, auto-fluorescent chemicals to identify senescence specific reporter. Senescent cells were generated by long-term in vitro culture of primary MSCs, and qualified by assessing growth kinetics, expression of ISCT marker panel, tri-lineage differentiation potential, β-galactosidase staining, and senescence associated secretory phenotype (SASP). Senescent MSCs and their isogenic, proliferative populations were screened to identify fluorescent probes for specific detection of senescent MSCs. The specificity of the senescent probe (SenezRed) was established by positive and negative correlations with conventional beta-galactosidase staining, and the expression of proliferative marker Ki67, respectively. The senescent probe exhibited specificity in detecting senescent human MSCs from 10 different sources, namely human bone-marrow, adipose tissue, umbilical cord and fetal tissues. Quantifying fluorescent intensities of probe-stained live MSC cultures by imaging or flow cytometry, demonstrated higher probe staining with long-term culture, which correlated with slower population growth kinetics with concomitant increase in beta-galactosidase staining. Importantly, the identified senescent cell population showed 2 to 5 fold higher production levels of TNF-alpha, IL-6, MMP-1 & 2, TIMP-1 and CXCL-5 indicative of the SASP. As the probe was found to predominantly localise within mitochondria, senescence associated loss of mitochondrial membrane potential might account for probe's binding specificity. We report a simple, rapid and quantitative method for analysis of senescence for manufacturing MSC therapeutics.

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T-2142

TERATOSCORE 2.0: ASSESSING TUMORIGENIC AND DIFFERENTIATION POTENTIAL OF HPSCS USING TERATOMA-DERIVED RNA-SEQ

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Teratoma formation is the gold standard assay for testing the capacity of human pluripotent stem cells (hPSCs) to differentiate into all embryonic germ layers. This is usually achieved qualitatively using standard evaluation of stained teratoma histological sections. We have previously developed an alternative, quantitative method called TeratoScore to evaluate teratoma composition using microarray expression data. As microarray is being rapidly replaced by high throughput RNA sequencing, we now adapted TeratoScore to utilize RNA-seq data derived from teratomas. Our new method identifies contribution of a dozen different tissues from the three germ layers, ectoderm, mesoderm and endoderm, as well as an extraembryonic tissue, the placenta. Differences identified by the TeratoScore in teratoma tissue-composition were validated using conventional histological staining. Furthermore, the sensitivity of RNA-sequencing allowed us to extend TeratoScore to identify foci of undifferentiated or primitive cells within some of the tumors, suggesting that their cells of origin harbor malignant potential. Together, the new TeratoScore allows a coherent analysis of teratomas and provides insights to cells ability to differentiate and their tumorigenic potential.

T-2144

SCALING UP A CHEMICALLY-DEFINED AGGREGATE-BASED SUSPENSION CULTURE SYSTEM FOR NEURAL COMMITMENT OF HUMAN PLURIPOTENT STEM CELLS

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The demand of high cell numbers for applications in cellular therapies and drug screening requires the development of scalable platforms capable to generating highly pure populations of tissue-specific cells from human pluripotent stem cells. This work describes the scaling-up of an aggregate-based culture system for neural induction of human induced pluripotent stem cells (hiPSCs) under chemically-defined conditions. Since initial cell density and aggregate size have an important impact in the expansion and commitment of these cells into a particular lineage, a combination of non-enzymatic dissociation and rotary agitation was successfully used to produce homogeneous populations of hiPSC aggregates with an optimal (140 µm) and narrow distribution of diameters (coefficient of variation of 21.6%). Scalable neural commitment of hiPSCs as 3D aggregates was then performed in 50 mL spinner flasks, and process optimization using a factorial design approach was developed involving parameters such as agitation rate and seeding density. We were able to produce neural progenitor cell cultures, that at the end of a 6-day neural induction process contained less than 3% of Oct4-positive cells and that, after replating, retained more than 60% of Pax6-positive neural cells. Furthermore, after scalable differentiation, hiPSC-derived neural progenitors still retained their multipotent potential, being able to give rise to neuronal and glial cells. The results presented in this work should set the stage for the future generation of a clinically relevant number of human neural progenitors for transplantation and other biomedical applications using totally controlled, automated and reproducible large-scale bioreactor culture systems.

T-2146

APPLICATION OF ATMOSPHERIC PRESSURE DURING CULTURE PROMOTES NEURAL PROGENITOR AND ADULT NEURONAL CELL DIFFERENTIATION IN IPSCS

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Induced pluripotent stem cells (iPSCs) can be used for autologous regenerative medicine to treat a multitude of health conditions, including debilitating injuries and genetic diseases. In order for iPSCs to be suitable for clinical use it is critical to optimize re-programming and differentiation conditions to consistently achieve functional and genetic attributes for target cells of interest, and in cell quantities that are therapeutically relevant. Recent studies have highlighted the significance of environmental factors such as oxygen concentration and pressure in stem cell culture; however, none have evaluated the combined effect of these factors for manipulating stem cell state. Previously we have shown increased efficiency in somatic cell reprogramming to iPSCs through cell culture in low oxygen and high pressure. Here we aimed to build on these previous works and demonstrate the relevance of a combination of hypoxia and pressure in directed differentiation in iPSCs using a custom bioreactor technology. We have developed a primary cell culture bioreactor, the AVATAR™, to provide tunable control of the microenvironment ex vivo and aimed to use this capability to optimize iPSC culture and directed differentiation. We used AVATAR™-defined settings for oxygen concentration (1-5%) and atmospheric pressure (0-5 PSI) and then assessed the impact of these conditions on stem cell state by evaluating expression of pluripotency-associated genes by RT-qPCR and immunofluorescence staining, whole-transcriptome sequencing, and directed differentiation into all three germ layers. Preliminary data of gene expression analysis indicates that iPSCs cultured in 2-5 PSI exhibit a spontaneous downregulation of pluripotency-related genes and upregulation of genes involved in neural induction. Differentiation studies subsequently showed long term culture with pressure substantially increased neural progenitor cell generation via PAX6 and neural cadherin (CDH2) expression and consequently allowed more efficient differentiation of iPSCs towards neurons and astrocytes. Our findings suggest that physiologically relevant oxygen and pressure are important factors in stem cell fate and can be useful stimuli for controlling and driving differentiation towards neural cell lineages.

T-2148

PRE-LOADING GUIDE RNAS INCREASES THE FREQUENCY OF TARGETED GENETIC MODIFICATIONS IN MOUSE EGGS

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Parthenogenetically activated eggs (parthenotes) develop to blastocysts whose inner cell masses are a source of pluripotent stem cells with potentially enhanced ease of genetic manipulation because of the single copy of each chromosome. Eliminating B2M, a protein essential to expression of the major histocompatibility complex on the cell surface, could give rise to hypoimmunogenic stem cells. Simultaneously eliminating CCR5, the receptor for HIV, could give rise to hypoimmunogenic stem cells also resistant to HIV infection. To make this clinically important possibility feasible with human eggs, the efficiency of targeted genetic modification of unfertilized eggs needs to be improved over published reports of approximately 10% of mouse eggs. Using mouse eggs as a model system, we have examined 3 strategies of microinjecting single guide RNAs (sgRNAs) and Cas9 enzyme into the pronuclei of unfertilized mouse eggs activated by strontium. The first strategy employed 2 sgRNAs targeting CCR5 plus 1 sgRNA targeting B2M, all individually pre-incubated with Cas9 to form the standard sgRNA/Cas9 complex. The second strategy pre-loaded the sgRNAs into the eggs by incubating them in culture medium supplemented with the 3 sgRNAs, followed by micro-injection of Cas9 enzyme. The third strategy pre-injected the three sgRNAs into pronuclei, followed by a second injection of Cas9 enzyme. Fertilized eggs were included in some experiments as controls. Analysis of early cleaving eggs by PCR amplification of target region, followed by RFLP and sequencing, demonstrated 75-95% of them remained wild type for both genes following the first strategy. Using different concentrations of sgRNAs or Cas9 protein from 200nM to 400nM did not improve the frequency of mutations. The second strategy, preloading the eggs with sgRNAs before injecting Cas9, decreased the number of wild type eggs to 33-37%. The third strategy, microinjecting pronuclei first with sgRNAs followed by Cas9 microinjection an hour later, also decreased wild type eggs to 33-38%. Results from these experiments suggest that the efficiency of Cas9-sgRNA mediated cleavage of target genes in mouse parthenotes can be increased approximately 4-fold either by pre-incubating or pre-injecting sgRNAs followed by Cas9.

T-2150

INTRODUCING THE STEM CELL HUB: THE CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE'S (CIRM) CENTER FOR EXCELLENCE IN STEM CELL GENOMICS (CESCG) DATA COORDINATION AND MANAGEMENT CORE (DCM)

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Stem cell genomics is a fast-expanding division within stem cell biology. The California Institute for Regenerative Medicine (CIRM) has initiated the Center for Excellence in Stem Cell Genomics (CESCG) as an innovative push towards accelerating stem cell therapeutic treatments. Within the CIRM CESCG, biomedical experiments are being developed which help us understand stem cell biology from the ground up. Information captured about these biomedical experiments, or data about the data, is generally referred to as metadata. This information may include details such as the sample name, location within the body the sample is from, and how it was prepared for sequencing. At the CIRM CESCG Data Coordination and Management Core (DCM), we relate metadata to sequence data to allow researchers to learn more about how stem cells react in a variety of disease models, treatment settings, differentiation and more. These data sets cover a large variety of sequencing assays, including a vast amount of single cell data. Researchers can compare experiments with our visualization tools, using metadata terms to color and arrange figures as a way of understanding which genes are driving stem cell actions.

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T-2152

RELATIVE FREQUENCIES OF RECURRENT ACQUIRED KARYOTYPIC ABNORMALITIES IN HUMAN PLURIPOTENT STEM CELLS FROM 2009 TO 2016

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Human pluripotent stem cells (hPSC) acquire karyotypic abnormalities during culture. Whole and partial gains of chromosomes 1, 8, 12, 17, 20 and X are recurrent aberrations; the only common loss is in the long (q) arm of chromosome 18. In order to determine the prevalence of each of these recurrent genetic changes, we compared the relative frequencies of each abnormality over the course of nearly eight years (from January 2009 to November 2016) during which approximately 7300 hPSC cultures were karyotyped. We assembled a dataset of 1189 hPSC cultures that had G-banded karyotypes with recurrent abnormalities. Due to the fact that some specimens contained multiple abnormalities, there are 1327 data points. We demonstrate that relative frequencies of recurrent acquired karyotypic abnormalities have dynamically changed over time. Three striking trends emerged: 1.) an increasing frequency of chromosome 1 long (q) arm gains; 2.) an increasing frequency of chromosome 20q gains; and 3.) a decreasing frequency of chromosome 12 short (p) arm gains. Gains of chromosome 20q have a dramatic increase in prevalence, ranging from 6% of all recurrent abnormalities observed in 2009 to a high of 49% in 2014. No gains of chromosome 1q were observed in 2009 but in 2016 they comprised a total of 27%. Gains of chromosome 12p declined from 60% to only 21% over the same period. The temporal changes are shared between both embryonic and induced pluripotent stem cells. As the methods for the derivation and culture of hPSC have changed through the years, these findings call for further research into factor(s) that may be responsible for altering their genomes.

T-2154

ROBUST DIFFERENTIATION PROCEDURES INTO CARDIOMYOCYTES OR ENDOTHELIAL CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS AND THEIR CHARACTERIZATIONS

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Induced pluripotent stem (iPS) cells are cultured on mouse feeder cells in many cases and then differentiated into various kind of cells. Since the feeder cell is uncontrollable factor and affect to the iPS cell culture and its differentiation, many feeder-free medium have been already developed. However, iPS cells cultured by most of them do not show similar features to cells by on-feeder, in the point of view of the undifferentiated state indicated by Tra1-60 expression and SSC pattern. DEF-CS™ culture medium can be used for culturing iPS/ES cells without mouse feeder cells (feeder-free) and the cultured cells show high Tra-1 expression and high SSC pattern similar to on-feeder cultured iPS cells. Because the condition of iPS cells as raw material is very important for stable and efficient differentiation, we used DEF-CS™ to make master cell bank (MCB) and working cell bank (WCB) of iPS cells. Then, we have developed robust differentiation procedures into cardiomyocytes or endothelial cells from iPSC-WCB. We tested for their reproducibility and differentiation efficiency of each procedure to obtain cardiomyocytes or endothelial cells in more than 10 rounds of experiments. For cardiomyocyte production, over 95% of cTnT-positive cells were obtained in all experiments. Then, we confirmed their predicted responses to various ion channel blockers in cell product (MiraCell™ Cardiomyocytes (from ChiPSC12)) produced by the established differentiation procedure. For endothelial cell production, we could also obtain over 95% of CD31/CD105/CD144 positive cells in all experiments. Furthermore, iPS cell-derived endothelial cells (MiraCell™ Endothelial Cells (from ChiPSC12)) produced by our procedure showed ehdothelial cell function such as tube formation and acetylated LDL uptake. Taken together, we could develop robust two differentiation systems into cardiomyocytes or endothelial cells from iPS cells. The robustness in both differentiation procedures is considered to result from the quality of iPSC MCB / WCB produced by DEF-CS™ culture system.

T-2156

SUPERIOR CLONING EFFICIENCY OF HUMAN PLURIPOTENT STEM CELLS CULTURED IN A XENO-FREE AND DEFINED CULTURE MEDIUM, STEMFIT®

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Human pluripotent stem cells (hPSCs) hold great promise for the study and treatment of human diseases because they can propagate unlimitedly and give rise to every cell type in the body. Recent advances in genome editing technology of hPSCs have allowed for the generation of more accurate human disease models with specific genetic alterations or correction of mutations in the mutant human induced pluripotent stem cell (hiPSC) lines. During genome editing experiments, it is important to isolate and expand hPSCs to obtain edited clonal cell lines. However, many hPSC lines grow too poorly from single cells. We have developed a defined and xeno-free culture medium, StemFit®, for feeder-free culture of hPSCs. Using StemFit®, in combination with extracellular matrices such as recombinant laminin or Matrigel®, hPSCs can be easily and stably passaged as dissociated single cells for long periods without any karyotype abnormalities. Here, we evaluated the cloning efficiency of hPSCs cultured in different media including StemFit®. Several hPSC lines cultured in StemFit® showed superior cloning efficiency both on laminin and Matrigel® matrices by serial dilution in 96 well plates. Even when cells cultured in other medium were passaged into StemFit® directly, dissociated cells could form colonies with high efficiency. All of the clones succeeded in expansion and maintained high expression of pluripotency markers. These results suggest that a feeder-free culture system using StemFit® is an optimal platform for genome editing experiments followed by human disease modeling research.

T-2158

THE MINIMUM INFORMATION ABOUT A STEM CELL EXPERIMENT (MISCE) TO CONSISTENTLY REPRESENT STEM CELL RESEARCH DATA

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The promise of stem cell therapies has generated an ecosystem of experiment data and hypotheses in regenerative medicine. To make progress in translating the proliferating variety and volume of data, representation standards are required to ensure accurate interpretation of experiment data between independent investigators. Consistent representation is one of the major challenges in ensuring that stem cell experiment data can be translated into clinical value. Thus, minimum data standards for stem cell experiments will pave the way for consistent, unambiguous and insightful interpretation of stem cell experiment information. Here we present the Minimum Information about a Stem Cell Experiment (MISCE) standard, which proposes the minimum information required to report a stem cell experiment. MISCE framework has been developed based on the planned process hierarchy in the Ontology for Biomedical Investigations (OBI). Semantic data models for Biomaterial Transformations, Assays, and Data Transformations were generated to extract data fields and mapped to relevant ontologies to control data values, which consistently represent the entire stem cell experiment workflow to provide meaningful information. MISCE framework has been implemented into Stem Cell Hub (SCHub) architecture, as the data warehouse for the California Institute of Regenerative Medicine (CIRM) Center of Excellence in Stem Cell Genomics (CESCG) projects. MISCE has not only refined the data architecture of the SCHub for backend developers, but also improved data interpretation for stem cell researchers; as tested in a variety of stem cell related projects with CIRM. The adoption of MISCE by the research community at large will facilitate consistent stem cell data interpretation and independent data validation.

Funding Source: California Institute or Regenerative Medicine (CIRM); Center of Excellence in Stem Cell Genomics (CESCG)

T-2160

DEVELOPMENT OF A ROBUST NEXT GENERATION FEEDER-FREE PLURIPOTENT STEM CELL MEDIUM

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Culture systems for pluripotent stem cell (PSC) expansion enable generation of a nearly unlimited pool of cells for downstream differentiation, disease modeling, drug discovery, and therapeutic applications. While a number of PSC feeder-free medium systems exist, there are many challenges encountered by stem cell scientists across the PSC workflow. Here we sought to improve the robustness and versatility of traditional PSC culture medium systems by utilizing several rounds of Definitive Screening and Custom Design DOEs to identify and optimize critical medium components. Through assessment of over 100 different formulations, an optimum medium composition was identified which provides compatibility across the PSC workflow from somatic cell reprogramming, PSC expansion, downstream differentiation, as well as providing support in gene editing applications. This system additionally provides versatility, allowing for every-other-day or weekend-free feed schedules and compatibility with a broad range of passaging reagents and matrices. We demonstrate that this system maintains normal PSC properties, including (1) expression of canonical pluripotency markers, including SOX2, SSEA4, Tra1-60, OCT4, and Nanog, (2) maintains trilineage differentiation potential, and (3) exhibit normal karyotype over long-term passaging. Together this system provides a robust next-generation stem cell medium system for today's PSC workflow needs.

T-2162

PULSED FOCUSED ULTRASOUND PRETREATMENT FOR ACCELERATING MESENCHYMAL STEM CELL MIGRATION AFTER IN VIVO TRANSPLANTATION

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Mesenchymal stem cells (MSCs) have shown considerable promise in the treatment of various diseases. The secreted trophic and immunomodulatory cytokines derived from MSCs, i.e. MSC secretome, have been used as regenerative medicine. However their therapeutic efficacy is often limited by the insufficient homing of systemically administered cells or undesirable side effects at sites. For stem cell therapy to be considered for clinical use for diseases, there needs to be functional improvement. We focused on MSC regulation for therapeutic efficacy and its mediators by modulating pulsed focused ultrasound (pFUS). MSCs were isolated from rat femur and tibial bones. The characteristics of MSCs were demonstrated by immunophenotyping. MSCs were plated in 24-well plate at 2 x 104 cells, and then we operated the in vitro scratch assay, mimicking the effects of cell-matrix and cell-cell interactions on cell migration. The pFUS parameters were: 0 ~ 20 W acoustic powers, 30 kHz frequency, burst modulation for 2 min, and 5% duty cycle. To analyze the change of cytokines in MSCs, we used cytokine array kit (customized). The cultured MSCs expressed CD44, CD90, CD105, and CD106. FACS analysis showed that cells were more than 90% positive on the MSC marker CD90. The cell migratory effect of pFUS groups (10~50% acoustic power) was increased as determined by the in vitro scratch assay, compared to pFUS non-treated group. However, there was no effect on cell death (apoptotic or necrotic cell death). The pFUS also increased expression of several cytokines related to cell homing or adhesion. Especially, monocyte chemoattractant protein (MCP)-1 and -2, known as regulator of migration and infiltration of monocytes/macrophages, were increased by pFUS stimulation. pFUS could potentially enhance therapeutic efficacy of MSCs to target homing by establishing local chemoattractant and trophic factors of cells toward local tissue. This may ultimately improve viability and flexibility of stem cell local therapy for several diseases. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2015R1C1A1A02037693).

Funding Source: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2015R1C1A1A02037693).

T-2164

QUANTIFICATION PERFORMANCE OF NON-INVASIVE LC-MS/MS ANALYSIS AND EVALUATION OF UNDIFFERENTIATED STATE OF HUMAN IPS CELLS

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To contribute to development and commercialization of regenerative medicines and drug discovery, we have aimed to establish a method for the evaluation of undifferentiated state of PSCs without cell disruption. We have developed a simultaneous analysis method for 95 compounds in basal medium and secreted metabolites using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Last year, we have reported that some biomarker candidates in cell culture supernatant identified by non-invasive LC-MS/MS analysis can be used for the evaluation of undifferentiated state of PSCs. However, it is generally known that LC-MS/MS quantitative results are influenced by matrices such as other metabolites and salts. Additional research was required to evaluate the influence of matrices on LC-MS/MS quantification performance before practical use of the biomarkers. hiPS cells (PFX#9) were maintained in TeSR-E8 medium on vitronectin, and three germ layer differentiation (endoderm, mesoderm, or ectoderm) were initiated by adding appropriate cytokines or chemicals into medium from 24 hours after seeding. Culture supernatants were collected every 24 hours and used as the samples for LC-MS/MS. Concentration of the biomarker candidates in each medium sample was quantified by two different calibration methods, external standard method and standard addition method. Standard addition method was expected to minimize matrix effects because the target compounds were spiked into the samples to generate calibration curves. Our results showed that the concentrations of target compounds obtained by the two calibration methods were nearly same, and the time-course profile on concentration of biomarker candidates was similar between the two calibration methods. These results indicate the external standard method provides reliable quantitative performance and matrix effects on the LC-MS/MS analysis are negligibly small in our application. Our LC-MS/MS method has the potential to be an effective means to evaluate the undifferentiated status of PSCs without cell disruption.

T-2166

EFFICIENT GENE EDITING OF HUMAN IPSCS USING DRUG SELECTION CASSETTE-FREE HOMOLOGY ARM

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Application of gene editing technology for iPSCs have been widely used to establish reporter cell lines, and drug selection-cassettes were frequently used to isolate recombined iPSCs. However, for gene editing around first ATG of coding DNA sequence or editing using single strand DNA (ssDNA) homology arm, drug selection cassette-free insertion vectors are preferable. To estimate frequencies of homologous recombination in iPSCs after the digestion by CRISPR/Cas9, we designed a reporter construct intending insertion of a YFP variant Venus followed by P2A sequence into around first ATG of human SOX2 gene, which is strongly expressed by iPSCs. To enrich iPSCs that were transiently transduced Cas9 and homology arm vectors, we used pSpCas9(BB)-2A-Puro (pX459) V2.0 as the Cas9 vector and proceeded short puromycin selection from 24 h until 60 h after electroporation. A week after the electroporation, fluorescence by Venus expression was successfully observed under microscope. Interestingly differentiated cells lost the fluorescence, consistent with the loss of SOX2 expression by differentiated cells. Flow cytometry analysis indicated the frequencies of homologous recombination in iPSCs after enrichment by 36 h puromycin was 16.5%. Next we investigated the frequencies of recombination with ~70 base pairs ssDNA homology arm. To estimate the frequencies of recombination we inserted XhoI sites at the center of homology arm. XhoI digestion after PCR amplification around the target sequence estimalted that the frequencies of recombination was ~20% using enrichment by puromycin. In fact, we successfully obtained an iPS clone harboring XhoI sites at the intended site after single cell cloning of iPSCs. Thus, the frequencies of recombination in iPSCs are higher than expected, and gene editing using drug selection cassette-free homology arm is a practical choice.

T-2168

PRODUCTION OF BILLIONS OF HUMAN INDUCED PLURIPOTENT STEM CELLS AS CELL-ONLY AGGREGATES IN SINGLE-USE STIRRED SUSPENSION BIOREACTORS

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The production of human induced pluripotent stem cells (hiPSCs) in high quantities that are sufficient for cell-based therapies and cell-loaded implants through standard adherent culture is not feasible and suffers from a lack of scalability. To overcome some of these limitations, a promising approach is to culture hiPSCs in suspension. In this study, stirred suspension culture vessels with volumes between 50 and 3000 mL were investigated for their compatibility with the expansion of hiPSCs inoculated as a single cell suspension, without supportive additives such as extracellular matrix or microcarriers. The simple and robust 2-phase process reported here first generates hiPSC aggregates of 324 ± 71 µm diameter in 7 days in 125 mL spinner flasks. These are subsequently enzymatically processed into a single cell suspension for inoculation into a 3000 mL bioreactor, finally yielding hiPSC aggregates of 198 ± 58 µm after 7 additional days. In these culture vessels, hiPSCs can be propagated as aggregates for more than 40 days in suspension, maintain an undifferentiated state as assessed by the expression of pluripotency markers TRA-1-60, TRA-1-81, SSEA-4, OCT4, and SOX2, are capable of differentiating into cells of all three germ layers, and can be directed to differentiate into specific lineages such as cardiomyocytes. Up to a 16-fold increase in hiPSC quantity at the 100 mL volume was achieved, corresponding to a fold increase per day (FIPD) of 2.28; at the 1000 mL scale, an additional 10-fold increase was achieved. Taken together, we expanded 16 × 10^6 hiPSCs into 2 × 10^9 hiPSCs in 14 days, for an FIPD of 8.93. This quantity of hiPSCs readily meets the requirements of cell-based therapies and brings their clinical potential closer to fruition.

Funding Source: The work is supported by a grant of the German Excellence Initiative to the Graduate School of Life Sciences, University of Wuerzburg, as well as funds from the Deutsche Forschungsgemeinschaft (DFG; ED79/4).

T-2170

PAIN RESPONSES IN HUMAN IPSC-DERIVED SENSORY NEURONS USING MEA SYSTEM

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Dorsal root ganglion (DRG) sensory neurons are pain-related neurons and have a variety of sensory receptors that are activated by chemical, thermal, and mechanical stimuli. Establishment of pharmacological assay in pain research and drug screening is important issue. In addition, human induced pluripotent stem cell (hiPSC)-derived sensory neurons may be effectively used for drug discovery and toxicity testing. The purpose of this study was to evaluate the physiological responses aginst pain-related molecules and temperature in cultured sensory neurons using multi-electrode array (MEA) system. Rat DRG neurons and hiPSC-derived sensory neurons (Axol) were cultured on MEA chips, and the extracellular signals in spontaneous firings and evoked responses by chemical and thermal stimuli were obtained by the MEA system. To investigate pain responses, we administered capsaicin, menthol, and allyl isothiocyanate (AIT) , and changed temperature from 30 to 46 ℃. After 2 days of rat DRG neurons culture on the MEA, we observed spontaneous activities and chemical responses. Addition of the capsaicin, menthol and AIT induced significant changes of the firing rate and concentration-dependent responses. We also found the difference of response speed and duration depending on the type of molecules, and that DRG neurons were classified into 27 patterns based on responses against 3 molecules. Furthermore, temperature elevation increased the number of firings and it showed the largest increase at 43 degrees. Moreover, we also detected the responses of capsaicin, menthol, AIT, and temperature changes in cultured human iPSC-derived sensory neurons, and confirmed the expression of Nav1.7, TRPM8, and TRPA1 receptors. We confirmed that the typical response of DRG neurons can be easily obtained using MEA system, and found that time variation of responses are dependent on the type of pain-related molecules, and that there are a lot of type neurons from electrophysiological responses of pain-related molecules. These results suggested that electrophysiological measurements in DRG neurons using a MEA system may be beneficial for clarifying the functions of DRG neurons in pain research and for drug screening applications.

T-2172

PATHOGEN REDUCTION THROUGH ADDITIVE-FREE UV-C IRRADIATION RETAINS THE OPTIMAL EFFICACY OF HUMAN PLATELET LYSATE FOR THE EXPANSION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS

Viau, Sabrina[1], Chabrand, Lucie[2], Eap, Sandy[2], Lorant, Judith[3], Rouger, Karl[3], Goudaliez, Francis[4], Sumian, Chryslain[4] and Delorme, Bruno[2]
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We recently developed and characterized a standardized and clinical grade human Platelet Lysate (hPL) that constitutes an advantageous substitute for fetal bovine serum (FBS) for human mesenchymal stem cell (hMSC) expansion required in cell therapy procedures, avoiding xenogenic risks (virological and immunological) and ethical issue. Because of the progressive use of pathogen reduced (PR) labile blood components, we evaluated the impact of the novel procedure THERAFLEX UV-Platelets for pathogen reduction on hPL quality (growth factors content) and efficacy (as a medium supplement for hMSC expansion). This technology is based on short-wave ultraviolet light (UV-C) and has the main advantage not to need the addition of any photosensitizing additive compounds (that might secondary interfere with hMSCs). We applied THERAFLEX UV-Platelets procedure on fresh platelet concentrates (PCs) suspended in platelet additive solution and prepared hPL from these treated PCs. We compared the quality and efficacy of PR-hPL with the corresponding non-PR ones. We showed no impact on the content in 5 cytokines tested (EGF, bFGF, PDGF-AB, VEGF and IGF-1) and a significant decrease in TGF-beta1 (-21%, n=16, $p < 0.01$). We performed large scale culture of hMSCs during 3 passages and showed that hPL or PR-hPL at 8% triggered comparable hMSC proliferation than FBS at 10% plus bFGF (n=3). Moreover, after proliferation of hMSCs in hPL or PR-hPL containing medium, their profile of membrane marker expression, their clonogenic potential and immunosuppressive properties (inhibition of T-cell proliferation) were maintained, in comparison with hMSCs cultured in FBS conditions. We quantitatively compared the potential to differentiate in adipogenic and osteogenic lineages of hMSCs cultured in parallel in the 3 conditions and showed that they remained also identical. In conclusion, we demonstrated the feasibility to use UV-C treatment to subsequently obtain pathogen reduced hPL, while preserving its optimal quality and efficacy for hMSC expansion for cell therapy applications.

Funding Source: Judith LORANT received financial support from French government (National Research Agency), Nantes Métropole and the Région Pays de la Loire.

FRIDAY, JUNE 16, 2017

POSTER SESSION III-ODD
18:00 – 19:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

F-1001

REGULATION OF BONE MARROW NICHE FOR TREATING BONE DISEASE IN DIABETES

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Osteoporosis (OP) and subsequent bone fracture are serious complications of type 1 diabetes mellitus (T1DM). Accumulated evidence has shown that T1DM have a negative effect on bone tissue. The mechanism for T1DM leading to low bone mass has not been clarified yet. However, most studies suggest that impaired bone formation has been proposed as a major factor. Current T1DM therapy such as continuous subcutaneous insulin infusion and transplantation of pancreas or islet cells cannot avoid the biomechanical deterioration of bone. To find the most appropriate treatment for OP in T1DM patients is an urgent issue. Amid advances in understanding the complexity of marrow environment and its role in the regulation of bone remodeling process, the role of fat, which is abundant marrow component in the adult bone, is still unclear in this process. Our preliminary results showed that increase of blood glucose and dramatic bone loss in femur of streptozotocin (STZ)-induced T1DM rats, which were alleviated by transplantation of Wharton's jelly mesenchymal stem cells (WJ-MSCs). In STZ-induced T1DM rats, increase of bone marrow adiposity was found. These findings raised the question that does the transplanted Wharton's jelly MSCs in STZ-induced T1DM rats homing to bone marrow and differentiate into osteoblasts? Is the increase of bone marrow adipocyte responsible for bone loss in T1DM? In this study, WJ-MSCs were transduced with luciferase (WJ-MSC-Luc)

by lentiviral vector. We traced their bone marrow distribution after transplantation and examine whether they are able to promote bone formation. 8-week-old S.D. rats with STZ treatment were used. Four weeks after the onset of diabetes, the diabetic rats were injected with WJ-MSC-Luc or normal saline. Results showed that STZ induced increase of blood glucose level in S.D. rats. Increased of bioluminescenc was detected in WJ-MSCs transduced with luciferase. In STZ+MSC group, decrease of blood glucose and leptin were found. Interestingly, the blood osteocalcin and adiponectin level were increased in these rats. In conclusion, this study provide mechanism of MSC differentiation into osteoblasts and adipocytes in bone marrow and explore pathogenesis and treatment for diabetes-associated bone loss.

Funding Source: MOST 105-2314-B-010-010-MY3

F-1003

TRANS-DIFFERENTIATION OF HUMAN WHARTON JELLY DERIVED MESENCHYMAL STEM CELLS INTO NEUROECTODERMAL CELLS

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Owing to the constrains associated with the isolation and long term maintenance of human neuroectodermal cells, scientists have started investigating for the alternate source of cells that can be used to generate neuroectodermal cells. Of all the other possible adult source cells, mesenchymal Stem Cells (MSCs) are found to be of great hope because of hypoimmunogenic, immunomodulatory, and transdifferentiation property. Here we have successfully generated neuroectodermal cells from Human Wharton jelly derived MSCs. These MSCs were extensively characterized based on its morphology, various gene expression profiles (RT-PCR, Immunocytochemistry, FACS and Western blot) and their multi lineage differentiation potential. These cells were found to express ACTA2, vimentin and fibronectin 1, the common genes associated with mesenchymal lineage. These cells also express SOX2 and POU5F1, the pluripotency genes. Apart from CD73, CD90, CD105 and CD146, they express SSEA-4. Unlike BM-MSCs, these cells were negative for CD271. As canonical Wnt is highly active in these MSCs, treatment with FGF2 and EGF lead to the formation of neuro-spheres like 3-dimentional bodies of sizes ranging from 200 to 600 micron. Further analysis of these cells confirmed drastic decrease in the expression of MSCs related genes with concomitant expressing of SOX1, the important transcription factor involved in neural fate determination. Expression of PAX6, musashi RNA binding protein 1 and nestin was also increased manifold in these differentiated cells as compared with MSCs. We also observed that these spheroid bodies express neural receptor NTRK1 and NTRK3 along with neuronal transcription factor like Neurod1, Neurod3 and Neurog2. The expression of SOX1, SOX2, PAX6, Nestin & Musashi were also confirmed by Immunocytochemistry.

Funding Source: Core Grant of National Institute Of Immunology, India.

ADIPOSE, MUSCULOSKELETAL, AND CONNECTIVE TISSUE

F-1005

OPTIMIZED APPROACHES FOR CONVERSION INDUCED MESENCHYMAL STEM CELLS FROM HUMAN SOMATIC CELLS WITH SMALL MOLECULES

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Human mesenchymal stromal/stem cells (MSCs) are hopeful in regenerative medicine and cell therapies for their multipotency and immune-modularity without risk of tumorigenesis. We have recently demonstrated that human dermal fibroblasts can be directly converted to functional MSC-like cells (induced MSCs, iMSCs) by a footprint-free method based on mixed small molecules within 6 days. However, the efficiency with an average conversion rate of 38% defined by marker expression remained room for improvement. Herein, we further screened several new combinations based on adding promising chemicals to the newly identified essential cocktail. So far, we have boosted the iMSC populations up to 70%. The optimized protocol implies a potential value for clinical application and cell fate modeling research by saving labor and time consumption of cell sorting and simplifying composition of the cocktail. Furthermore, we currently test and modify the cocktails working on the different source of human somatic cells including peripheral circulating blood cells (PBMC) and keratinocytes to evaluate the approach assisting for the larger clinical application. Of note, the cocktail shows a promising performance of improving the function of MSCs. Therefore, we treated the senescence or aging primary human MSCs with the cocktail to successfully generate iMSCs with higher differentiation ability compared to the parental cells. Overall, our discovery in this study and our previous findings reveal a new strategy to enrich more iMSCs by small molecule fine-tuning.

Funding Source: This work was supported by Academia Sinica, the National Health Research Institute (NHRI-EX105-10515SI), and the Taiwan Ministry of Science and Technology (105-2628-B-002-021-MY3, 104-0210-01-09-02, 105-2320-B-001-026-MY2).

F-1007

BMI1 INHIBITS SENESCENCE AND ENHANCES THE IMMUNOMODULATORY PROPERTIES OF HUMAN MESENCHYMAL STEM CELLS VIA THE DIRECT SUPPRESSION OF MKP-1/DUSP1

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For the application of mesenchymal stem cells (MSCs) as clinical therapeutics, the regulation of cellular aging is important to protect hMSCs from an age-associated decline in their function. In this study, we evaluated the effects of hypoxia on cellular senescence and the immunomodulatory abilities of hUCB-MSCs. Hypoxic-cultured hUCB-MSCs showed enhanced proliferation and had increased immunosuppressive effects on mitogen-induced mononuclear cell proliferation. We found that BMI1, a member of the polycomb repressive complex protein group, showed increased expression in hypoxic-cultured hUCB-MSCs, and the further knock down of BMI1 in hypoxic cells induced decreased proliferative and immunomodulatory abilities in hUCB-MSCs, along with COX-2/PGE2 down-regulation. Furthermore, the expression of phosphorylated p38 MAP kinase increased in response to the over-expression of BMI1 in normoxic conditions, suggesting that BMI1 regulates the immunomodulatory properties of hUCB-MSCs via p38 MAP kinase-mediated COX-2 expression. More importantly, we identified BMI1 as a direct repressor of MAP kinase phosphatase-1 (MKP-1)/DUSP1, which suppresses p38 MAP kinase activity. In conclusion, our results demonstrate that BMI1 plays a key role in the regulation of the immunomodulatory properties of hUCB-MSCs, and we suggest that these findings might provide a strategy to enhance the functionality of hUCB-MSCs for use in therapeutic applications.

Funding Source: The Bio & Medical Technology Development Program of the National Research Foundation (NRF)(No. 2012M3A9C6049716), the Research Institute for Veterinary Science, Seoul National University.

F-1009

MOUSE ADIPOSE TISSUE-DERIVED STEM CELLS COULD BE AN IDEAL SOURCE FOR IN UTERO THERAPEUTIC REPAIR OF FETAL BRAIN DYSFUNCTION

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Congenital neurological disorders are caused by an abnormal development and a dysfunction of the brain that begins from the fetal period, resulting in physical symptoms. Several experimental cell therapies for the fetus have been applied, but there is less evidence which uses adipose tissue derived stem cells (ADSCs) as a cell source. The purpose of this study is to define the neural commitment of ADSCs in vitro and analyze their distribution and tissue integration after in utero ADSC transplantation. ADSCs were isolated from the inguinal adipose tissue of 8-week-old mice by enzymatic digestion. To generate neural differentiation, ADSCs were stimulated with retinoic acid, bFGF, forskolin and GGF5. To verify the ADSCs neurogenesis, neural markers were examined by immunocytochemistry and the neurogenesis-related gene expressions were examined by RT-qPCR. To clarify whether transplanted ADSCs could populate in a brain tissue, ADSCs obtained from CAG-EGFP Tg mice were injected into fetal-brain ventricle at E14.5, then GFP positive cells were confirmed by fluorescence microscopy at E19.5 and the neural differentiation of these cells were investigated by immunohistochemistry. ADSCs have a variable appearance having neurite morphology including bulbous cytoplasm with elongated cell process like axon and/or dendrites after neural induction. The cells expressed neural markers, GFAP, S100 and Tuj1 by immunocytochemistry. Also, GFAP and NSE mRNA were increased after the induction stimulation. Transplanted GFP-positive ADSCs grew tightly around the ventricle and migrated deep into the brain. The transplanted ADSCs were able to differentiate into neuron-like and astrocyte-like cells. Our data suggests that ADSCs could be a promising cell source of neural regeneration as neural stem cells.

Funding Source: Grant-in-Aid for Research Activity start-up

F-1011

DEVELOPING AN IPSC-BASED MODEL TO UNDERSTAND A GENETIC CAUSE OF PREMATURE CARTILAGE DEGENERATION IN CHILDREN

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Novel strategies are needed when standard approaches fail to uncover the mechanism by which a mutation causes disease. This is the case for the severe, degenerative joint disease that occurs in children with Progressive Pseudorheumatoid Arthropathy of Childhood (PPAC), caused by loss of function mutations in the gene encoding Wnt-inducible signaling pathway protein 3 (WISP3). PPAC becomes symptomatic in children between the ages of 3 and 8, and then rapidly progresses to end-stage articular cartilage failure that is indistinguishable from end-stage osteoarthritis. Consequently, these children require total hip and knee replacement surgeries in their teenage years. Because pre-symptomatic cartilage tissue from children with PPAC is unavailable (i.e., it is unethical to invasively collect "unaffected" joint tissue from children), and mice lacking Wisp3 show no sign of disease, we developed a human induced pluripotent stem cell (iPSC) model of PPAC in order to gain insight into its pathobiology. We generated iPSCs from 5 patients with PPAC, and using the directed differentiation methods developed by our lab, we have differentiated PPAC iPSCs into two different cartilage lineages, articular and growth plate cartilage. In an unbiased approach, we have begun to analyze the molecular, biochemical, and biomechanical profiles of these in vitro-derived cartilage tissues in order to identify similarities among cartilages derived from PPAC-iPSCs that collectively differ from those derived from WISP3-sufficient iPSCs. The knowledge we gain about mechanisms that lead to cartilage failure in this novel iPSC-based model system will benefit children born with PPAC, and will likely point to new approaches for protecting cartilage from damage associated with more common forms of joint degeneration and osteoarthritis.

Funding Source: Charles H. Hood Foundation Child Health Research Award (A.M.C)

F-1013

COMPARISON OF HUMAN PLATELET LYSATE AND SILK IN THE GENERATION OF A FUNCTIONAL 3-D HUMANIZED FAT PAD IN-VITRO AND IN-VIVO UTILIZING HUMAN ADIPOSE STROMAL VASCULAR FRACTION

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Adipose tissue, and specifically liposuction aspirate, is a rich and convenient source for stem/progenitor cell populations. As such, adipose tissue has emerged as an attractive source for regenerative medicine therapeutic approaches such as stem cell-seeded grafts. One such component of liposuction aspirate, the stromal vascular fraction (SVF), has demonstrated particularly robust regenerative potential in damaged tissues through paracrine signaling and differentiation mechanisms. In combination with biocompatible materials, SVF makes a strong candidate for inclusion in medical tissue grafting applications. In this study, we seeded SVF onto either solid silk fibroin (SF) grafts or into media supplemented with human platelet lysate (hPL) and compared each SVF-loaded graft's ability to promote the generation of a functional 3-dimensional humanized fat pad in-vitro and in-vivo. Traditional silk-based biomaterials offer exceptionally low immunogenicity and degradation rates, and their mechanical properties add to their ease of implantation. Additionally, media supplemented with 7.5% hPL has been shown to acquire a gel-like consistency after addition of SVF, making the graft material very favorable to injection. Cell-seeded grafts (SF-SVF and hPL-SVF) were cultured in-vitro and assayed for biological properties including human SVF cell growth and colony formation, immunophenotype, adipogenic differentiation, and functional lipolysis and glucose uptake. SF-SVF and hPL-SVF grafts were also implanted into the dorsal cutaneous fat pads in mice and allowed to grow for 8 weeks. The resulting fat pads were assayed for histological analysis, RNA content, and immunophenotype. Both in-vitro and in-vivo, hPL-SVF grafts demonstrated enhanced proliferation and showed potential for the formation of a humanized 3-D adipose depot. As measured by flow cytometry, qPCR, and confocal microscopy, hPL-SVF grafts demonstrated enhanced persistence and proliferation over SF-SVF grafts.

Funding Source: This work is supported by NSF grant: 1621439 entitled "Tissue engineered human fat on a chip - a novel tool for metabolic drug discovery."

F-1015

HUMAN MESENCHYMAL PROGENITORS FROM NON-SKELETAL SOURCES DISPLAY LIMITED CHONDROGENIC DIFFERENTIATION

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Stromal cells from multiple tissues are currently used in numerous clinical trials for cartilage and bone regeneration despite lack of evidence for engraftment and robust differentiation. Predictive potency assays are urgently needed for selecting reliable cell products for therapeutic application. We hypothesized that a direct comparison of established positive and negative control samples combined with stringent application of molecular and histo-morphological analyses is sufficient to qualify the chondrogenic potential of different stromal progenitors. Clinically tested cell sources of mesenchymal stem/progenitor cells (MSPC) from adult bone marrow (BM), white adipose tissue (WAT) and umbilical cord (UC) were compared to mature chondrocytes (hCh; positive control) and fibroblasts (negative control) in a 3D chondrogenesis potency assay in vitro. Cell identity, purity and viability were tested by flow cytometry. Differentiation was measured by Movat's pentachrome, Alcian/Fast Red and Safranin O/Fast Green histochemistry and additionally monitored by marker gene RT-qPCR and cartilage protein expression. All cells tested displayed the canonical mesenchymal phenotype. Purity was >98% and viability >90%. Cartilage disk formation rate was 100% in BM-MSPC & hCh vs. 0% in WAT- and UC-MSPC and fibroblasts. BM-MSPC and hCh formed significantly larger cartilage templates, built significantly more proteoglycan per mg total protein and showed significantly higher expression of chondrogenic genes (ACAN, COL2) during 3D chondrogenesis compared to all other cell types. Only BM-MSPC showed strong collagen X expression in vitro corresponding to their bone formation potential in vivo. WAT-MSPC expressed more aggrecan and collagen II compared to UC-MSPC and fibroblasts but significantly less than BM-MSPC and hCh. Histochemistry-based Bern scoring correlated with these results. These results support the notion that a natural skeletal regeneration potential appears to be restricted to skeletal progenitors and their mature progeny. Our data favor collagen II-forming hCh for cartilage and collagen X/II-forming BM-MSPC for bone regeneration. Clinical trials testing non-skeletal cells (e.g. WAT, UC) for skeletal regeneration need to be evaluated with particular caution regarding efficacy and safety.

Funding Source: EU Horizon 2020 grant agreement No. 668724 to D.S.

F-1017

HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS ALLEVIATE ATOPIC DERMATITIS BY REGULATING B LYMPHOCYTE AND MAST CELL

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Mesenchymal stem cell (MSC) has been applied for the therapy of allergic disorders due to its beneficial immunomodulatory abilities. However, their effects and mechanisms can be altered according to the source of cell isolation and the route of administration. We sought to investigate the safety and the efficacy of human adipose tissue-derived MSCs (hAT-MSCs) in mouse atopic dermatitis (AD) model and to determine the distribution of cells after intravenous administration. Murine AD model was established by multiple treatment of Dermatophagoides farinae. AD mice were intravenously infused with hAT-MSCs and monitored for clinical symptoms. To determine the mechanism, co-culture of B lymphocytes with hAT-MSCs was performed to determine the change in B cell proliferation and maturation. The administration of hAT-MSCs reduced the gross and histological signatures of AD as well as serum IgE level. hAT-MSCs were mostly detected in lung and heart of mice within 3 days after administration and were hardly detectable at 2 weeks. All of fifty five mice administered with hAT-MSCs survived until sacrifice and did not demonstrate any adverse events. It turned out that hAT-MSCs significantly inhibited the proliferation and the maturation of B lymphocytes via cyclooxygenase (COX)-2 signaling. Moreover, mast cell (MC) degranulation was suppressed when hAT-MSCs were co-cultured. The intravenous infusion of hAT-MSCs can alleviate AD through the regulation of B cell function and suppression of mast cell (MC) degranulation.

F-1021

PERCUTANEOUS DELIVERY OF HUMAN AMNIOTIC STEM CELL TREATMENT IN A NOVEL RABBIT MODEL OF DISC DEGENERATION

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Degenerative disc disease (DDD) is a leading cause of back pain. Advances in the treatment of DDD rely on reproducible animal models that recreate this condition. Current models are invasive, require an open approach to the disc space, and are associated with significant morbidity. The purpose of this animal study was to establish a reproducible model of DDD and administration of amniotic stem cells to the injured disc in rabbits using a percutaneous needle technique. We hypothesize that the proposed technique will effectively produce disc degeneration with minimal harm to the animal and that amniotic stem cell treatment will attenuate degenerative changes. Ten New Zealand white rabbits were used. Under anesthesia, rabbits were placed in a prone and slightly lateral position. Initial lateral images were obtained to ascertain L5-L6 disc levels. Under fluoroscopic guidance, an 18-gauge needle was inserted 2 cm to the right of the midline spinous process. The needle was slowly advanced at a 30 to 45 degree angle until it is adjacent to but not touching the vertebral body to prevent osteophyte formation. Anteroposterior views verified correct needle position into the L5-L6 nucleus pulposus. At 4 weeks post-injury, 4 of the rabbits were treated with 10 microliters of stem cell suspension into the disc space using a similar technique as described previously. X-rays were obtained weekly for 12 weeks to measure the disc height index (DHI). Repeated measures ANOVA was performed to trend the change in DHI over time. Data are reported in means ± standard error. DHI was 0.094 ± 0.006 at baseline, which decreased to 0.030 ± 0.003 at 4 weeks. In the control group, final DHI was 0.029 ± 0.001; in the treatment group, final DHI was 0.039 ± 0.002 (p=0.01). Our findings suggest the percutaneous approach using an 18-gauge needle produces an effective model of DDD in rabbits. Changes in DHI are evident as early as 1 week after injury with final DHI significantly greater in the treatment group in comparison to controls, although still less than 50% of baseline values. The injury and treatment procedures are minimally invasive, with low risk to the well-being of the animal. This experiment provides the impetus for future research into novel treatments of degenerative disc disease.

Funding Source: Nutech Medical, Birmingham, AL

CARDIAC TISSUE

F-1025

MEASURING THE CONTRACTILE FORCE OF HUMAN IPSC-DERIVED CARDIOMYOCYTES IN MEDIUM THROUGHPUT WITH CARDIAC MICROWIRES

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In collaboration with the Centre for Commercialization of Regenerative Medicine (CCRM, Toronto, Canada), we have developed a semi-automated analysis platform to measure the contractile force of cardiomyocytes embedded in an in vitro microtissue, referred to as a cardiac microwire. With the aim of accelerating evaluation of lead compounds in our heart failure programs, we have implemented a medium-throughput assay in which human iPSC-derived cardiomyocytes and cardiac fibroblasts are seeded into collagen and Matrigel-rich mixtures in 96-well plates; driven by spontaneous contractions, a 3-dimensional cardiac microtissue develops within each well over a 2 week period. Each microtissue wraps around flexible, PDMS posts, which deflect upon contraction of the microtissue; these contractions are recorded with video microscopy and subsequently analyzed to measure contractile force. Here we will discuss the advancements we have made to automate several steps of this experiment cycle, including cell seeding with liquid handling equipment, image acquisition via automated microscopy, and image analysis with custom-written scripts based on the generation of kymographs. Our current efforts are focused on assay optimization in order to detect increases and decrease in contractile force with positive and negative inotropes, respectively. In addition, we are exploring pacing of cardiac microwires via electrical stimulation in order to prevent chronotropic effects of select compounds from masking their inotropic effects. Here we will discuss assay optimization and present results from a panel of cardioactive inotropes in the cardiac microwire assay, and we will also address future goals to stimulate maturation of cardiac microtissues in an effort to better simulate physiological conditions.

F-1027

CHARACTERIZING THE CONTRIBUTION OF GENETIC BACKGROUND TO ELECTROPHYSIOLOGICAL TRAITS AT BASELINE AND AFTER RESPONSE TO CARDIO-ACTIVE DRUGS IN HUMAN IPSC-DERIVED CARDIOMYOCYTES

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Cardiovascular diseases are the leading cause of mortality in developed countries accounting for over 30% of reported deaths and are the result of both environmental and genetic factors. Drug treatments for many of these conditions often prove to be ineffective and/or produce adverse side effects exhibiting a high degree of inter-individual variability. Accordingly, such drug therapies have been known to cause acquired types of cardiovascular diseases, such as drug-induced arrhythmias (sudden cardiac arrest). While the growing popularity of personalized medicine aims to provide patient-specific treatments, one of the limitations in pushing this field forward is the inability to obtain human primary tissues (i.e. the heart) for study, especially with varied genetic backgrounds. Hence, we have generated a well characterized collection of induced pluripotent stem cells (iPSCs) from 222 individuals [iPSCORE - iPSC Collection for Omic Research], developed methods of large-scale derivation and bio-banking of iPSC-derived cardiomyocytes, and are currently conducting in-depth molecular analysis of these cells. The 222 whole-genome sequenced individuals in iPSCORE includes twin pairs, extended families and some individuals with cardiovascular disease. For the current study, iPSC-derived cardiomyocytes with a variety of genetic backgrounds have been selected and are being subjected to high-throughput Microelectrode Array (MEA) analysis. These analyses will determine how genetic background contributes to electrophysiological phenotypes and associated drug-triggered responses. We focus on six different concentrations of cardio-active drugs, including Cisapride and Sotalol, examining iPSC-derived cardiomyocytes on the MEA at baseline and after response to drug administration.

F-1029

CARDIO PROGENITOR CELLS DERIVED FROM HUMAN IPS CLONES EXHIBIT HYPERSENSITIVITY TO DOXORUBICIN

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Doxorubicin (Dox) is a widely prescribed drug used in the treatment of many types of tumors. However, 3-8 % of patients receiving Dox will develop a cardiomyopathy. An in-vitro diagnostic tool able to determine Dox hypersensitivity could help prevent patients from developing future Dox-related complications. hiPSC-derived cardiomyocytes have been reported to predict patient susceptibility to Dox-induced cardiomyopathy. However, our data suggest that cells most sensitive to Dox treatment arise around the fifth day of differentiation (n=6, p=0.03). Flow and RT-PCR analyses performed in conjunction with our collaborators at the Mayo Clinic BioTrust demonstrate that these cells express genes associated with a cardio progenitor phenotype (Nkx2.5, GATA-4), show reduced expression of pluripotency and early mesoderm formation genes (POU5F1, SOX-1, SOX-2, T), and exhibit increased mitochondrial activity (CellROX, MitoSOX). To explore the mechanisms responsible for the Dox sensitivity in hiPSC-derived cardio progenitor cells, we performed RNAseq analysis on hiPSCs derived from Dox-exposed healthy controls (n=3) and Dox-induced cardiomyopathy (DIC) patients (n=3). hiPSCs were treated w/wo Dox for 48 hr. between D5-D8 of cardiac differentiation. The analyses revealed 1070 differential expressed genes (DEGs) in controls and 2004 DEGs in DIC patients. Of those genes, approximately 70% are novel transcripts. Biological function enrichment analysis revealed that the overlapping DEGs in both patient and control samples are mainly involved in apoptosis response to DNA damage, likely explaining the mechanism of Dox treatment. However, DEGs specific to DIC patients include regulators of hormone stimulus, cyclin-dependent protein kinase activity, membrane depolarization, endothelial cell migration, muscle stretch, and RNA polymerase II regulation. DEGs specific to the control samples primarily involved protein glycosylation, action potential, endothelial cell differentiation, cellular ion homeostasis, neuromuscular process controlling balance, cell adhesion, and transmembrane transport. Our ultimate goal is to develop a gene expression panel capable of predicting doxorubicin sensitivity, and secondarily, identify genes that could be targeted to alleviate Dox hypersensitivity.

Funding Source: Todd and Karen Wanek Family Program for Hypoplastic Left Heart Syndrome

F-1031

SELF-ASSEMBLY OF AN IN VIVO FUNCTIONAL HUMAN HEART GRAFT PATCH FROM PURIFIED VENTRICULAR PROGENITORS

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The generation of human pluripotent stem (hPSC) cell-derived ventricular progenitors and their assembly into a three-dimensional in vivo functional ventricular heart patch has long been an elusive goal. Herein, we report the large-scale generation of an enriched pool of hPSC derived ventricular progenitors (HVPs), which can expand, differentiate and self-assemble into a functional ventricular wall in vivo following transplantation. A two-step protocol can generate billions of pure HVPs, marked by Islet-1 (ISL1), creating new in vivo chimeric models of human cardiac muscle disease and a new paradigm of organ-on-organ cardiac tissue engineering.

F-1033

NITRIC OXIDE MODULATES POSTNATAL BONE MARROW-DERIVED MESENCHYMAL STEM CELL MIGRATION

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Nitric oxide (NO) is a small free-radical gas molecule, which is highly diffusible and can activate a wide range of downstream effectors, with rapid and widespread cellular effects. NO is a versatile signaling mediator with a plethora of cellular functions. For example, NO has been shown to regulate actin, the microfilament, dependent cellular functions, and also acts as a putative stem cell differentiation-inducing agent. In this study, using a wound-healing model of cellular migration, we have explored the effect of exogenous NO on the kinetics of movement and morphological changes in postnatal bone marrow-derived mesenchymal stem cells (MSCs). Cellular migration kinetics and morphological changes of the migrating MSCs were measured in the presence of an NO donor (S-Nitroso-N-Acetyl-D, L-Penicillamine, SNAP), especially, to track the dynamics of single-cell responses. Two experimental conditions were assessed, in which SNAP (200 μM) was applied to the MSCs. In the first experimental group (SN-1), SNAP was applied immediately following wound formation, and migration kinetics was determined for 24 hours. In the second experimental group (SN-2), MSCs were pretreated for 7 days with SNAP prior to wound formation and the determination of migration kinetics. The generated displacement curves were further analyzed by non-linear regression analysis. The migration displacement of the controls and NO treated MSCs (SN-1 and SN-2) were best described by a two parameter exponential functions expressing difference constant coefficients. Additionally, changes in the fractal dimension (D) of migrating MSCs were correlated with their displacement kinetics for all the three groups. Overall, these data suggest that NO may evidently function as a stop migration signal by disordering the cytoskeletal elements required for cell movement and proliferation of MSCs.

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F-1035

STABLY EXPRESSED APEX2 IDENTIFIES T-TUBULES AND DYAD FORMATION IN ENGRAFTED IPSC-DERIVED CARDIOMYOCYTES IN A MOUSE MODEL OF MYOCARDIAL INFARCTION

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Although studies have shown the potential of in vivo cardiac transplantation of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) in animal experiments, nano-structural confirmation of the engrafted iPSC-CMs including electron microscopy (EM) has not been accomplished, partly because identification of graft cells in EM has proven to be difficult. Moreover, immaturity of iPSC-CMs remains as a critical problem. Especially, excitation-contraction coupling is one of the fundamental properties of cardiomyocytes,

the absence of dyad formed between T-tubule and junctional portion of the sarcoplasmic reticulum is one of the major reasons of reduced coupling gains and arrhythmogenic risks after cardiac transplantation. With a new genetically encoded probe, the monomeric 28-kDa peroxidase reporter 2 (APEX2), which withstands strong EM fixation, cells and molecules are identified in EM and their nano-structures are confirmed in vitro. We tested whether nuclear targeted APEX2 could identify iPSC-CMs in host heart after long-term engrafting, and evaluated the engrafted iPSC-CMs in post-myocardial infarction using EM. We established human iPSC lines which stably expressed histone H2B-APEX2 (APEX2 iPSCs). After differentiating APEX2 iPSCs into CM in vitro, purified cells were transplanted into NOG mouse hearts with myocardial infarction by direct injections into the myocardium. APEX2 did not give significant influences on cardiac differentiation in vitro, and stably expressed in iPSC-CMs over 6 months in vivo. APEX2 reaction clearly identified engrafted APEX2 iPSC-CMs in EM surrounded by host CMs. The maturation of sarcomeric structure and mitochondria were evident, and T-tubules and dyads started to emerge in engrafted iPSC-CMs at 6 months after transplantation. We demonstrated that APEX2 is a versatile genetic reporter to trace cell fates in living animals over many months. We unequivocally demonstrated that T-tubules and dyads can be formed in iPSC-CMs after a substantially long period of engraftment. This method should be useful to many studies of stem cell-based cell replacement therapy, as it allows direct nano-scale structural characterization of engrafted cells in EM.

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ENDOTHELIAL CELLS AND HEMANGIOBLASTS

F-1037

DIFFERENTIAL EFFECTS OF SUPEROXIDE AND HYDROGEN PEROXIDE ON IN VITRO ANGIOGENIC FUNCTION OF ENDOTHELIAL COLONY-FORMING CELLS

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Endothelial colony-forming cells (ECFCs) hold significant cytotherapeutic potential for ischaemic disease, capable of homing to sites of tissue ischaemia and integrating with host vasculature to promote angiogenesis and reperfusion. Emerging evidence supports a key role for reactive oxygen species (ROS), such as superoxide (O2-) or hydrogen peroxide (H2O2), in underlying angiogenic processes of endothelial cells, although their precise influence is not known. In order to study the effects of endogenous and exogenous ROS on the pro-angiogenic function of ECFCs, cells were isolated from umbilical cord blood and assessed in vitro under basal and pro-oxidative conditions using migration and tubulogenesis assays. Exposure to exogenous O2-, stimulated via the PKC activator PMA (100nM for 16h), increased cell migration (wound closure: control 23.3.±7.9, PMA 51.1±19.3 arbitrary units; n=6, $P < 0.001$) and tubulogenesis (branch length: control 6.8±0.9, PMA 8.4±1.4 arbitrary units; n=6, $P < 0.05$) in a O2--dependent manner, indicated by attenuation of these effects by the O2- scavenger, PEG-SOD. Furthermore, incubation of cells with PEG-SOD under basal conditions significantly reduced both migration (wound closure: control 23.3.±7.9, SOD -3.8±10.2 arbitrary units; n=6, $P < 0.01$) and tube formation (branch length: control 8.7±2.9, SOD 1.7±1.0 arbitrary units; n=6, $P < 0.001$) compared to control, highlighting the critical importance of endogenous O2- in ECFC angiogenic function. In contrast, stimulation with the H2O2 mimic, tert-butyl hydroperoxide (tBH, 100nM) resulted in a non-significant increase in migration, which was reduced below control levels by the H2O2 scavenger, catalase (wound closure: control 23.3±7.9, catalase 10.4±13.2 arbitrary units; n=9, $P < 0.05$), whilst in vitro tube formation was completely prevented in the presence of tBH. Taken together, these data indicate that ECFC pro-angiogenic function is enhanced by O2--mediated signalling, but inhibited by H2O2, highlighting ROS-specific effects which are likely to be complex and compartment-dependent. These findings may have significant implications for ECFC-based therapies for ischaemic disease and emphasise the importance of the oxidative microenvironment in modulating endothelial progenitor cell function.

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F-1039

MITOCHONDRIAL ABNORMALITIES ARE RELATED TO THE DYSFUNCTION OF CIRCULATING ENDOTHELIAL-COLONY-FORMING CELLS IN MOYAMOYA DISEASE

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Moyamoya disease (MMD) is a unique cerebrovascular disorder characterized by the progressive occlusion of the bilateral internal carotid artery. Endothelial colony–forming cells (ECFCs), which were previously termed endothelial progenitor cells, play an important role in the pathogenesis of MMD. In this study, we performed morphological and functional studies of the mitochondria of ECFCs from MMD patients to present new insights into the pathogenesis of MMD. The morphology of the ECFCs from the MMD patients and normal controls was examined under both a transmission electron microscope and a confocal laser scanning microscope. The oxygen consumption rates (OCRs), mitochondrial membrane potentials (MMPs), intracellular Ca2+ concentrations, mitochondrial enzyme activities, and reactive oxygen species (ROS) levels were measured. The functional activity of the ECFCs was evaluated by capillary tube formation assay. The ECFCs from the MMD patients displayed a disrupted mitochondrial morphology, including a shorter and more circular shape. The mitochondria of the ECFCs from the MMD patients exhibited functional abnormalities, which were assessed as an increased OCR and increased intracellular Ca2+ concentration. Moreover, the ECFCs from MMD patients showed increased ROS levels. Interestingly, treatment with a ROS scavenger not only rescued the mitochondrial abnormalities, but also restored the angiogenic activity of the ECFCs from the MMD patients. The mitochondria of the ECFCs from the MMD patients exhibit morphological and functional abnormalities compared to normal ECFCs. This finding suggests that the mitochondrial abnormalities may have a possible role in the pathogenesis of MMD.

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HEMATOPOIESIS/IMMUNOLOGY

F-1043

N6-METHYLADENOSINE EPITRANSCRIPTOMIC CONTROL OF HUMAN ERYTHROID LINEAGE SPECIFICATION

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Regulation of mammalian erythropoiesis is governed by erythroid specification of the bi-potent megakaryocyte-erythroid progenitor (MEP) and the further maturation of its progeny into erythrocytes. While the stages of mammalian erythropoiesis are well characterized at the cellular level, only a handful of key molecular regulatory activities have been elucidated. To identify new regulators of erythropoiesis, we performed a genome-wide CRISRP-Cas9 screen in human erythroleukemia (HEL) cells for genes promoting expression of CD235A/GYPA, a prominent erythroid-specific cell surface marker, constitutively expressed on HEL cells. By screening for CD235A low expressing cells, we were able to identify known regulators of erythropoiesis including GATA1 and LMO2, in addition to several novel screen hits. Among the novel hits, we report the identification and validation of genes coding for the N6-methyladenosine (m6A) mRNA regulatory machinery, including METTL14, METTL3, WTAP, and YTDF2. We show that inhibition of these genes causes dramatic down regulation of erythroid-specific genes and upregulation of megakaryocyte/ platelet-specific genes in HEL cells. Critically, their inhibition in human hematopoietic stem and progenitor cells (HSPCs) causes complete ablation of the erythroid lineage from the MEP stage, with little effect on megakaryopoiesis or myeloid differentiation. Utilizing methylated-mRNA-IP-sequencing analysis in HEL cells, we identified m6A mRNA targets including ribosomal proteins and key hematopoietic transcriptional regulators, among them FLI1, GATA1 and LMO2. Unexpectedly, m6A mRNA deficient cells had few m6A mRNA targets with altered transcript levels, suggesting translational regulation. For this meeting, we will present the results of ribosome profiling analysis in m6A mRNA deficient cells and identify key translationally regulated m6A mRNA targets required for erythropoiesis. Taken together, our results suggest that epitranscriptomic regulation of m6A-containing mRNA plays a critical role in human erythroid lineage specification.

F-1045

MOSAIC MUTAGENESIS AND COLOR BARCODING REVEAL COMBINATIONS OF MUTATIONS THAT LEAD TO CLONAL HEMATOPOIESIS IN ZEBRAFISH

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Clonal hematopoiesis of indeterminate potential (CHIP) occurs when a hematopoietic stem cell (HSC) clone acquires an advantageous mutation and outcompetes normal clones. This state precedes and strongly predicts hematopoietic malignancies, including myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). It has been difficult to assess combinations of mutations that promote or cooperate in CHIP in a native setting. To follow output of HSC clones endogenously, we recently developed a marking strategy in which each HSC clone is labeled with a unique fluorescent hue using the transgenic line Zebrabow, and here have used the system to determine mutations that drive clonal expansion and those that participate in transforming clones to leukemia. To induce clonal hematopoiesis in zebrafish, we introduced combinations of somatic mutations found in human MDS and AML into Zebrabow embryos. When grown to adulthood, each zebrafish harbored unique sets of mutations associated with specific myeloid phenotypes. Mosaic mutations in five epigenetic factors — asxl1, ezh2, dnmt3a, dnmt3b, and tet2 — induced by CRISPR/cas9 resulted in the expansion of single color clones contributing >30% of granulocytes in zebrafish after 8 months post-fertilization (mpf). The majority of zebrafish exhibiting clonal expansion harbored frame-shift mutations in asxl1 with >25% variant allele frequency. This clonal expansion remained non-pathological up to 17 months, consistent with CHIP. We observed a significant expansion of progenitor populations in the marrow compared to controls. When mutations in asxl1 or tet2 were combined with overexpression of Jak2V617F or FLT3ITD, color dominance was apparent by 3 mpf. The dominance was associated with an expansion of progenitor or blast-like populations in the marrow and spleen at the expense of mature myeloid cell production. Our studies have successfully incorporated a unique color barcoding system to characterize clonal hematopoiesis and early stages of leukemic transformation driven by mosaic mutagenesis. Our findings lay the groundwork for a mutational 'code' that coordinates specific hematopoietic disease states.

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F-1047

ASCORBATE REGULATES HEMATOPOIETIC STEM CELL FUNCTION AND SUPPRESSES LEUKEMOGENESIS IN MICE

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Stem cell fate can be influenced by metabolite levels in culture but it is unknown whether physiological variations in metabolite levels within normal tissues regulate stem cell function in vivo. We developed a metabolomics method for analysis of rare cell populations isolated directly from tissues and used it to compare mouse hematopoietic stem cells (HSCs) to restricted hematopoietic progenitors. Each hematopoietic cell type had a distinct metabolic signature. Ascorbate levels were highest in HSCs and declined with differentiation. Depletion of ascorbate in mice increased HSC frequency and function as a result of reduced Tet2 function, a dioxygenase tumor suppressor. This allowed hematopoietic stem and progenitor cells with Flt3ITD leukemic mutations to outcompete wild-type cells in an ascorbate-depleted, but not in an ascorbate-replete, environment, and accelerated leukemogenesis by Flt3ITD; Tet2+/- cells. Ascorbate thus accumulates within HSCs to promote Tet function, limit HSC frequency and suppress leukemogenesis.

F-1049

GENERATION OF A NOVEL TARGETED BLOOD CELL ABLATION SYSTEM TO STUDY HEMATOPOIETIC STEM CELL-DRIVEN REGENERATION IN ZEBRAFISH (DANIO RERIO)

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Hematopoietic stem and progenitor cells (HSPCs) function in both steady state production and rapid regeneration of mature blood cells. Acquisition of these regenerative properties occurs during embryogenesis

but when and how are poorly understood. Given that regenerative potential diminishes with age, studying embryonic HSPCs within their biological niche may provide insight into how we can restore this capacity later in life. To study embryonic HSPC-driven hematopoietic regeneration, we developed a blood-specific inducible cell ablation zebrafish model. In this transgenic system, the draculin (drl) promoter drives expression of nitroreductase (NTR) (drl:CFP-NTR) only in blood cells. Exposure to the prodrug Metronidazole (MTZ) leads to ablation of only NTR-expressing cells. Co-expression analyses of drl:CFP-NTR with known blood cell markers revealed that drl:CFP-NTR is restricted to HSPCs (runx1+23:mCherry) and erythrocytes (gata1:dsRed). To delineate the regeneration potential of embryonic HSPCs, we exposed 2-day-old drl:CFP-NTR zebrafish embryos to a 24-hr MTZ treatment and evaluated recovery of drl+ HSPCs/progenitors and gata1+ erythrocytes over a 7-day period. drl+ cells begin to recover by 4 days post MTZ treatment, while gata1+ erythrocytes exhibit a delayed recovery at 6 days post treatment. Our findings suggest that HSPCs can respond to injury as early as 2 days of life and that the HSPC-driven regeneration of embryonic blood cells occurs in a hierarchical fashion, similar to that of the adult blood system. Overall, we demonstrate a novel quantitative method for in vivo real time monitoring of blood regeneration, which may be further manipulated in a drug screen to identify factors that regulate HSPC-driven blood regeneration.

F-1053

INITIATION OF DEFINITIVE ERYTHROPOIESIS BEFORE HEMATOPOIETIC STEM/PROGENITOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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The development of human erythroid cells has been routinely investigated by using models of adult hematopoiesis, while their initiation during embryonic/fetal stage is largely unknown. We have reported that the early erythroblasts derived from human pluripotent stem cells (hPSCs) initially endowed with definitive characteristics but still shared mesodermal and endothelial properties. These human early erythroblasts' progenitors were enriched in glycophorin A+ (GPA+) CD34+ (G+34+) fraction. The G+34+ cells appeared by day 5 (D5) in coculture with mouse aorta-gonad-mesonephros stromal cells (AGM-S3), much earlier than hematopoeitic stem/progenitor cells (HSPCs, CD34+CD45+) did, which were generated on D8 in coculture. Respectively, CD34 and GPA single positive cells appeared before G+34+ cells during the coculture, while the earliest G+34+ cells were exclusively generated from CD34+ fraction with endothelial potential. The G+34+ cells burst on D7 and peaked on D10 in the coculture. D7 G+34+ cells could be induced to differentiate to erythroid, myeloid and endothelial cells in secondary cultures. Colony assay showed that erythropoietic potential was highly enriched within sorted D7 CD43+G+34+ (43+G+34+) cells. 622 ± 29 colony forming units of erythrocyte (CFU-E) and 16 ± 10 colony forming units of granulocyte and monocyte (CFU-GM) were detected in 104 43+G+34+ cells, while D7 43-G+34+cells gave rise to little hematopoietic progenitors (32 ± 5 CFU-E and 14 ± 10 CFU-GM be detected in 104 cells). Different from D7's results, D10 43+G+34+ cells were of little endothelial potential and much lower erythropoietic potential. We found that inducible over-expression of GATA2 or GATA1 at proper time points could enhanced the generation of G+34+ cells respectively. And GATA1 promoted more 43+G+34+ cells that enriched with erythroid progenitors. Our study may highlight the understanding of definitive erythropoiesis before the generation of HPSCs during human embryogenesis.

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F-1055

TRACKING HEMATOPOIETIC PRECURSOR DIVISION EX VIVO IN REAL TIME

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Deciphering molecular mechanisms underlying self-renewal and differentiation of hematopoietic stem cells (HSCs) would facilitate bone marrow transplantation for treating diverse hematological diseases. Using a novel reporter of hematopoietic precursor Evi1-GFP, we track the division of hematopoietic precursors in culture in real time. First, we confirmed that Evi1-GFP is a faithful reporter of HSC activity and identified three dividing patterns of HSCs: symmetric renewal; symmetric differentiation; asymmetric dividing. Furthermore, we found that the cytokines and growth factors combination (STIF) promotes symmetric renewal whereas OP9 stromal cells balance symmetric renewal and differentiation of HSCs ex vivo. Interestingly, we found that Tet2 knockout HSCs underwent more

symmetric differentiation in culture compared to wild type control. Our study established a new system to explore the molecular mechanisms of the regulation of HSC fate ex vivo. The knowledge learned may contribute to the discovery of novel ex vivo expansion protocol of HSC in future.

Funding Source: NHLBI

F-1057

REGULATION OF NUTRIENT SENSING SIGNALING OF MTORC1 IN HEMATOTOPOIETIC SYSTEM

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Nutritional status can impact steady-state hematopoiesis, as observed in malnutrition or obesity, as well as be an important determinant in therapeutic settings, such as in hematopoietic stem cell (HSC) transplantation. Surprisingly, how information about changing nutrient levels is interpreted by hematopoietic cells has remained a largely unexplored avenue of therapeutically informative research. A major molecular sensor of cellular nutritional status is the mTOR. mTOR kinase is in a multiprotein complex along with the scaffolding protein RAPTOR (regulatory-associated protein mTOR), termed mTORC1, it can sense multiple upstream energy-status inputs, as well as non-nutritional signals. Once activated mTORC1 phosphorylates substrates that promote anabolic cellular processes, such as mRNA translation. mTORC1 is required for HSC regeneration under transplantation conditions and lineage-choice decisions. The Ras-related GTP binding protein (Rag) GTPases activate mTORC1 in response to nutritional input, specifically glucose and amino acids. However, the upstream inputs to mTORC1 governing hematopoiesis are not known. Further, the anabolic pathways governed by mTORC1 in these processes are also ill defined. To investigate the role of nutrient sensing signaling to mTORC1 in the hematopoietic system, we utilized Mx1Cre-mediated homozygous deletion of the RagA GTPase, which a core recruiter of mTORC1 to the lysosome post amino acid (AA) stimulation. RagA mutant's phenocopy loss of the mTORC1 component Raptor, resulted in mild pancytopenia, splenomegaly and monocytoid cell outgrowth. However, RagA loss did not impair HSC activity under stress conditions. While RagA-deficient HSCs were unresponsive to acute AA changes, they displayed compensatory basal upregulation of mTORC1 activity in response to serum factors, which allowed them to grow under stress conditions. Finally, we show that the chronic hyperactivation of mTORC1 by a gain of function RagA allele does not result in HSC exhaustion. Manipulation of the nutrient sensing arm of the mTOR pathway is therapeutically attractive in several disease states. Collectively, we predict lack of toxicity to normal HSCs after either inhibition or activation of the nutrient sensing arm of the mTORC1 pathway in vivo.

F-1059

ID1 ABLATION ENHANCES HEMATOPOIETIC STEM CELL SELF-RENEWAL AND SUPPRESSES HEMATOPOIETIC AGING BY REDUCING PROLIFERATIVE STRESS

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Inhibitors of DNA binding (Id) proteins are helix-loop-helix (HLH) transcription factors that lack a basic DNA binding motif. Id proteins (Id1-4) heterodimerize with class II HLH proteins, E proteins, and inhibit their ability to bind DNA, activate transcription and regulate proliferation and differentiation. As key regulators of E proteins, Id proteins have been implicated in the regulation of neural, epithelial, and hematopoietic stem cell (HSC) proliferation and self-renewal. Despite its low expression in HSCs under homeostatic conditions, inhibitor of DNA binding protein 1 (Id1) is induced in HSCs by pro-inflammatory cytokines that promote HSC proliferation and differentiation in vitro and in vivo, suggesting that Id1 may function during stress hematopoiesis. Id1-/- HSCs show increased self-renewal in serial bone marrow transplantation (BMT) assays, which is correlated with decreased cell cycling, proliferation, and DNA damage. Id1-/- HSCs show reduced mitochondrial biogenesis and stress, less ROS production and increased intracellular levels of reduced glutathione after BMT, demonstrating that Id1-/- HSC are more quiescent after BMT. Cytokines and other pro-inflammatory stimuli present in the bone marrow niche after γ-IR induce Id1 expression and proliferation in HSCs, suggesting that HSC exhaustion is mediated, in part, by chronic proliferative stress. Id1-/- HSCs are protected from other chronic proliferative stresses including chronic genotoxic and inflammatory stress, and aging. Thus, targeting Id1 inhibition may be therapeutically useful to improve HSC survival and function during BMT, chronic stress and aging.

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PANCREAS, LIVER, KIDNEY

F-1061

GLI-SIMILAR PROTEIN 3 (GLIS3) REGULATES SELF-RENEWAL AND DIFFERENTIATION IN ADULT MURINE PANCREATIC PROGENITOR-LIKE CELLS IN VITRO

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Adult pancreatic progenitor cells are a potential source of insulin-secreting beta-like cells for cell replacement therapy in diabetic patients. Recently, progenitor-like cells capable of self-renewal and multi-lineage differentiation in vitro were found in the adult murine pancreas and were termed pancreatic colony-forming units (PCFUs) due to their ability to give rise to colonies in a methylcellulose containing culture system. It was also shown that the Wnt agonist Rspondin-1 increased PCFU self-renewal. However, detailed mechanisms underlying PCFU self-renewal and differentiation remain unclear. RNA-seq analysis in our laboratory showed that PCFUs have a high expression of Gli-similar 3 (Glis3). Glis3 is a transcription factor that was shown to regulate pancreatic duct and endocrine development. Glis3 mutation leads to cystic ducts, a lack of certain endocrine cell formation (including beta cells) and in some cases a lack of exocrine development. These findings led us to hypothesize that Glis3 is important in regulating both self-renewal and differentiation of adult murine PCFUs in vitro. To assess self-renewal, PCFUs were obtained by sorting CD133(high)CD71(low) cells from the adult murine pancreas, infected with viral vectors containing shRNAs against Glis3 (shGlis3) or a control vector, plated in the presence of Matrigel and RSPO1, and serially dissociated and replated for 5 generations. We found that knockdown of Glis3 in PCFUs decreased their expansion, suggesting that Glis3 is required for RSPO1-induced self-renewal. Single-colony microfluidic qRT-PCR showed Wnt target genes were decreased in 3-week-old colonies treated with shGlis3. These results suggest that Glis3 may interact with Wnt signaling. Interestingly, we discovered that CD133 mRNA and protein were significantly decreased in colonies receiving shGlis3 compared to controls. This suggests that CD133, a stem cell marker for many adult organs, may participate in Wnt-Glis3 signaling. Knockdown of Glis3 in PCFUs plated in a differentiation medium containing laminin hydrogel resulted in decreased Insulin 2 gene expression in colonies, suggesting that Glis3 is important in the differentiation of PCFUs into beta-like cells. We conclude that Glis3 is important in the self-renewal and differentiation of adult murine PCFUs in vitro.

F-1063

MITOCHONDRIA TRANSFER OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS INHIBITS APOPTOSIS IN PROXIMAL TUBULAR EPITHELIAL CELLS IN DIABETIC NEPHROPATHY

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Although bone marrow-derived mesenchymal stem cells (BM-MSCs) are promising therapeutic tools for various diseases including diabetic nephropathy (DN), the mechanism is not known clearly. Recently, the phenomenon in which BM-MSCs transfer their own mitochondria (Mt) into recipient cells, called "Mt transfer", has been reported. Mt transfer results in initiation of stem cell differentiation, reprogramming of differentiated cells and functional recovery of degenerated Mt in recipient cells. Since no previous studies have shown the Mt transfer of BM-MSCs as a mechanism of therapeutic potentials for DN, we aimed to clarify the evidence with in vivo and in vitro experiments. Streptozotocin-induced diabetic mice (STZ mice) were used as a model of DN. In vivo studies, BM-MSCs in which endogenous Mt were labeled with DsRed2 (MtDsRed2-MSCs) were injected into STZ mice via the tail vein, and the kidney was analyzed morphologically at day 3 and 7 after cell administration. In vitro studies, we first co-cultured MtDsRed2-MSCs with primary proximal tubular epithelial cells (PTECs) isolated from STZ rats. Next, we isolated Mt from MtDsRed2-MSCs and added to culture supernatant of PTECs. The function of PTECs was evaluated by the expression of lotus tetragonolobus lectin (LTL), a marker of PTECs, and TdT-mediated dUTP Nick-End Labeling (TUNEL). The results showed that DsRed2 labeled Mt derived from intravenously injected MtDsRed2-MSCs were detected in proximal tubular epithelial cells of STZ mice at both day 3 and 7 after cell administration. In vitro experiments, we found the evidence of Mt transfer from MtDsRed2-MSCs into PTECs, as well as the transfer of isolated Mt directly into PTECs. Abnormal aggregation of LTL and the number of TUNEL-positive cells were inhibited in PTECs by co-culturing with MtDsRed2-MSCs or isolated Mt. This is the first study to demonstrate the Mt transfer from BM-MSCs to renal tubular epithelial cells in DN mice and the efficiency to lead the inhibition of apoptosis in PTECs. These results suggest that Mt transfer from BM-MSCs to recipient cells might be an important mechanism

for functional recovery of damaged cells caused by diabetes.

F-1065

STEATOGENIC COMPOUNDS INDUCE TRIGLYCERIDE ACCUMULATION BY MULTIPLE MECHANISMS IN HEPATOCYTE-LIKE CELLS GENERATED FROM HUMAN SKIN-DERIVED PRECURSORS

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Non-alcoholic fatty liver disease (NAFLD) ranges from reversible steatosis to severe, life threatening non-alcoholic steatohepatitis (NASH). Today's investigations of NAFLD and NASH rely mainly on animal models, which are not representative for the human situation. In addition, currently available human in vitro models do not adequately mimic the in vitro situation. Therefore, there is a high demand for a predictive, human-based in vitro system that accurately represents the molecular mechanisms involved in the progression of NAFLD. Human skin precursors (hSKPs) are multipotent stem cells that can be easily isolated from small human skin segments. These cells are able to convert into cells with hepatic characteristics (hSKP-HPC) upon exposure to hepatic growth factors that play a role during liver development. hSKP-HPC exposed to a variety of different steatogenic compounds (e.g. tetracycline, sodium valproate, oleic acid and insulin) accumulate lipids intracellularly. This study investigates the molecular mechanisms involved in this steatotic response. Multiple mechanisms play a role in the accumulation of intracellular lipids, namely (i) fatty acid uptake, (ii) de novo fatty acid synthesis, (iii) β-oxidation and (iv) lipoprotein secretion in the form of very low-density lipoprotein (VLDL). To evaluate these different modes of action, we analyzed the modulation of expression of key genes in both hSKP-HPC and HepaRGTM cells exposed to steatogenic compounds. hSKP-HPC showed an increased de novo lipogenesis (upregulation of SCD1), a decrease of fatty acid β-oxidation (downregulation of ACADSB and CPT-1) and a decrease in the secretion of VLDL (downregulation of APOB). HepaRGTM cells exposed to the same steatogenic compounds showed a decrease of β-oxidation and a decrease in the secretion of VLDL, but no induction of de novo lipogenesis. Our study concluded that human skin stem cell-derived hepatic cells can elucidate multiple mechanisms of action involved in the onset of NAFLD and can therefore be of interest for potential use in preclinical in vitro screening of novel molecules for the treatment of NAFLD.

F-1067

MESENCHYMAL STEM CELLS STABLY TRANSDUCED WITH A DOMINANT-NEGATIVE INHIBITOR OF CCL2 AMELIORATE CHRONIC LUNG DISEASE AND PULMONARY HYPERTENSION

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Recently, studies on mesenchymal stem cells (MSCs) for the treatment/prevention of chronic lung disease (CLD) are emerging. Although macrophages participate in the development of CLD, MSCs only weakly modulate macrophage function. 7ND-MSC is an MSC that secretes 7ND, a dominant-negative inhibitor of CCL2, which is a chemokine to promote the activation of macrophages. In this study, we clarified the treatment/preventive effect of 7ND-MSC for CLD model rats. This animal experimental protocol was approved by the Institutional Review Board of our institution. We established a CLD model by exposing Wistar/ST rat pups to 80% oxygen from birth to 15 days. At postnatal 5 days, 7ND-MSC (7ND group), control MSC (C group) (1 × 10^5 cells), or vehicle (acetic acid Ringer's solution) (V group) were administered via the right external jugular vein. After 15 days of exposure to hyperoxia, tissue volume density and medial wall thickness was evaluated with paraffin sections. In addition, we measured the expression of various mRNAs (including IL-6 and CCL2) in lung tissue and performed differential counts of bronchoalveolar lavage fluid (BALF) and blood cells. Tissue volume density was higher and medial wall thickness was lower in the 7ND group than those in the C group and the V group (tissue volume density: 7ND group 30.20 ± 0.70%; C group 26.22 ± 0.95%; and V group 26.16 ± 0.56%; $p < 0.01$. medial wall thickness: 7ND group 24.00 ± 0.48%; C group 29.72 ± 0.53%; and V group 34.23 ± 2.25%; $p < 0.001$), indicating that 7ND-MSC had a treatment/preventive effect for alveolar destruction and pulmonary hypertension. Compared to in the V group, IL-6 mRNA and CCL2 mRNA in the 7ND group were significantly decreased, but not in the C group (IL-6/GAPDH: 7ND group 5.86 ± 1.26; C group 12.44 ± 2.12; V and group 16.59 ± 4.68; $p < 0.01$, CCL2/GAPDH: 7ND group 2.15 ± 0.26; C group 3.39 ± 0.31; and V group 4.21 ± 0.94; $p < 0.001$). The number of white blood cells in BALF and blood was significantly smaller in the 7ND group than in the V group, indicating that 7ND-MSC had a more potent anti-inflammatory effect. In conclusion,

the present study showed the treatment effect of 7ND-MSC in CLD model rats and that its effect was stronger than that of control MSCs.

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F-1069

IDENTIFICATION OF MICRORNA-DEPENDENT GENE REGULATORY NETWORKS DRIVING HUMAN PANCREATIC ENDOCRINE CELL DIFFERENTIATION

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An abundant literature has demonstrated that microRNAs (miRNAs), a type of small non-coding RNA molecule that typically regulates the expression levels of hundreds of genes, can drive cell lineage specification in various tissues. Although evidence indicates that miRNAs may play key roles in pancreas development and endocrine function, the role of miRNAs in pancreatic endocrine cell differentiation has not been systematically explored. To address this, we performed genome-wide small RNA sequencing analysis on pancreatic progenitor cells differentiated in vitro from human embryonic stem cells and primary human endocrine cells isolated from whole human islets. This analysis revealed several miRNAs that are highly expressed in endocrine cells compared to pancreatic progenitor cells. Employing gain-of-function experiments, we identified four miRNAs that can repress a large number of genes that are lowly expressed in human islets. These miRNAs were found to repress genes encoding cell-type-specific transcription factors known to regulate endocrine cell differentiation as well as cell cycle regulators. This knowledge about miRNA target genes in conjunction with HITS-CLIP data allowed us to construct an integrated miRNA-gene regulatory network of endocrine cell differentiation. Our integrated analysis indicates a key role for the identified miRNAs in establishing a transcriptional landscape that promotes the differentiation of pancreatic progenitor cells into endocrine cells. This study not only sheds light on the mechanisms that underlie human endocrine cell differentiation, but also has important implications for devising improved protocols for producing replacement beta cells for diabetes cell therapy.

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F-1071

DUAL RECONSTITUTION MODEL OF THE LIVER AND HEMATOPOIETIC SYSTEM USING MOUSE FETAL LIVER CELLS

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The immune system plays an important role in the pathological process of virus-/drug-induced hepatitis. To replicate the human immunoresponse to hepatitis in an animal model, some studies reported the dual humanization model of the liver and hematopoietic system in a mouse by transplanting human hepatocytes and hematopoietic stem cells (Washburn et al., 2011, Gutti et al., 2014, Wilson et al., 2014). However, it is difficult to avoid hepatocyte rejection because of allogeneic reactions caused by immune cells that are differentiated from hematopoietic stem cells derived from another donors. In the developmental stage, the fetal liver contains both liver and hematopoietic stem cells in human and mouse. Thus, establishing a syngeneic dual reconstitution model is possible if it was generated from identical fetal liver cells. Therefore, we examined the dual reconstitution of the liver and hematopoietic system by transplanting hepatoblasts and hematopoietic stem cells isolated from the mouse fetal liver. In the mouse development, AFP+/ALB+ hepatoblasts accounted for only 2.5% of embryonic day 12.5 (E12.5) fetal liver, and those cells expressed cell surface maker, E-cadherin (Nierhoff et al., 2005). We then purified E-cadheirin expressing hepatoblasts from E12.5 green fluorescence protein transgenic mouse fetal liver and transplanted in uPA-NOG mouse liver. As a result, the reconstitution of the liver was significantly improved compared with the transplantation of crude fetal liver cells. For establishing the dual reconstitution model, we are examining the transplantation of both E-cadherin+ and hematopoietic stem cells that were isolated from GFP mouse fetal liver. Our results would provide useful information for establishing a syngeneic reconstruction model in dual humanized mice with human fetal liver cells.

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EPITHELIAL TISSUES

F-1075

DROSOPHILA INTESTINAL STEM CELLS EXPRESS MULTIPLE AUTOCRINE AND PARACRINE LIGANDS TO MAINTAIN HOMEOSTASIS

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Epithelial homeostasis requires the precise balance of epithelial stem/progenitor proliferation and differentiation. While many of the signaling pathways that regulate epithelial stem cells have been identified less is known about their targets or crosstalk between them. The stem cells of the Drosophila midgut exhibit similar rules of cell fate to mammalian epithelial stem cells of the epidermis, esophagus and intestine, and are regulated by many of the same conserved signaling pathways, making them an excellent simple model for the regulation of stem cell fate. Here we use gene expression profiling by targeted DamID to identify the stem/progenitor specific transcription and signaling factors in the Drosophila midgut. Many signaling pathway components, including ligands of most major pathways, exhibit stem/progenitor specific expression and have regulatory regions bound by both cell-type specific transcription factors and effectors of extrinsic pathways. In addition to previously identified stem/progenitor-derived ligands we show that both the insulin-like peptide dilp6 and TNF ligand egr are specifically expressed in the stem/progenitors and regulate normal tissue homeostasis. We propose that the integration of extrinsic and cell-type specific regulatory factors allows stem and progenitor cells to maintain a homeostatic micro-environmental niche through the expression of autocrine and paracrine factors.

Funding Source: This work was supported by an HFSP long term fellowship (DPD), work in the Perrimon lab is funded by NIH and HHMI.

F-1079

AUTOPHAGY-DEPENDENT INCREASE OF TUFT CELLS DURING COLITIS

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Inflammatory Bowel Diseases are a group of autoimmune disorders that destroy the intestinal epithelium and sometimes they are associated to colorectal cancer development. The mechanism driving this process is not well understood. However, previous reports showed that the proinflammatory cytokines IFNγ and TNFα decrease proliferation of epithelial cells and increase apoptosis, both, through the activation of PI3K/Akt signaling pathway, affecting the intestinal homeostasis and leading to epithelial barrier lost. Despite the absence of cell proliferation during colitis, we have detected an increase in the number of a specific epithelial cell population, the DCAMKL1+ cells; surprisingly, LPS triggers a similar effect. DCAMKL-1, also known as Dclk1, is a common marker for Tuft cells. The role of this cell population in the intestinal epithelium remains unknown but their presence has been associated to inflammatory mucosal response initiation and cancer development. Interestingly, DCAMKL1 has also been proposed as a marker for quiescent stem cells and its ablation impairs epithelial repair. We sought to understand the mechanism behind the increase of Tuft cells during intestinal inflammation finding that autophagy inhibition prevents this augmentation. Moreover, the Disease Activity Index increased in colitic mice where autophagy was suppressed. Therefore, our study suggests a role of autophagy in development of Tuft cells and the importance of this population in the maintenance of intestinal homeostasis and the tissue regeneration during colitis.

F-1081

SOX9 IS A KEY TRANSCRIPTION FACTOR OF SALIVARY GLAND DEVELOPMENT

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The salivary glands arise as a thickening of the primitive oral epithelium, invaginate downwards into mesenchyme, and continually develop by branching morphogenesis. There have been several studies to investigate the molecular mechanism of salivary gland development, which reported the involvement of various growth factors and extracellular matrix in these processes. However, little is known about the exact molecular mechanism during the earliest stages of salivary gland formation. Our aim was to identify transcription factors restricted to the presumptive site of salivary gland development on oral epithelium. In this study, we isolated oral epithelium without association with salivary gland formation, oral epithelium above submandibular gland rudiment, and submandibular gland rudiment from E12.5(?) mouse using laser capture microdissection, respectively and their gene expression profiles generated by RNA-seq were compared. Consequently, both expressions of Sox9 and Sox10 in submandibular gland rudiment were higher than those in oral epithelium without association of salivary gland development. Importantly, Sox9 expressed at not only submandibular gland rudiment but also oral epithelium above submandibular gland rudiment. In addition, to determine whether Sox9 is necessary for organogenesis, we suppressed Sox9 expression in the organ cultures of embryonic mouse submandibular gland using siRNA. Sox9 knockdown inhibited such organogenesis of salivary gland as branching formation, suggesting that Sox9 possibly regulate submandibular gland development. Next, to identify genes regulated directly by Sox9, Sox9 ChIP-seq was performed in embryonic submandibular gland. Several genes including Etv5 and Barx2 were identified as Sox9 target genes. These data indicate that Sox9 may be involved in early salivary gland development through directly regulating the target genes such as Etv5 and Barx2.

F-1083

GLOBAL HISTONE METHYLATION REGULATES ADULT HAIR FOLLICLE STEM CELL (HFSC) HOMEOSTASIS

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Elucidating the molecular mechanism of adult mammalian stem cell (SC) fate decision is critical for tissue stem cell homeostasis and regeneration, yet is poorly understood. In mouse hair follicle stem cells (HFSCs), cell fate decisions to self-renew or differentiate occur during the quiescent stage of the hair homeostatic cycle, when we found reduced histone methylation levels. To examine the physiological relevance of histone H3 tri-methylation levels, I maintained the histone H3 methylation levels high using demethylase inhibitors or transgenic mice with epithelial expression of different combination of the HMTs Setd1b (H3K4me3 HMT), Suv39h2 (H3K9me3 HMT) and Ezh2 (H3K27me3 HMT) in mouse qHFSCs. I found delayed hair cycle progression, as judged by HF morphology, decreased proliferation of the progenitor cells, and delayed hair growth. For both approaches I will examine the ability of HFSCs to differentiate, which I predict would be impaired if low levels of histone methylation are important for qHFSC plasticity. Moreover, I will also examine mRNA levels using RNA-seq to identify potential mechanisms responsible for these phenotypes.

F-1085

ESTABLISHMENT OF A HUMAN EPITHELIAL STEM CELL MODEL FROM NASOPHARYNX TISSUE FOR THE ANALYSIS OF VIRAL DISEASE MECHANISMS

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Various microbial pathogens target the upper respiratory tract causing different clinical symptoms with varying severity in the nasopharynx. For instance, influenza virus (IAV), adenovirus (HAdV) and Epstein-Barr virus (EBV) are known to infect, among others,

the nasopharynx leading to epithelial damage and viral nasopharyngitis. On the other hand, in the context of EBV infection, long-term consequences might include the development of nasopharyngeal carcinoma (NPC). To date, modelling disease mechanisms in the human nasopharynx is limited to the usage of difficult to obtain ex vivo organ cultures combined with quantity-limited primary nasopharynx cell culture models or immortalized nasopharyngeal tumour cell lines. Each system harbours specific advantages but also disadvantages and only the human ex vivo organ system can sufficiently recapitulate infection mechanisms. However, limitations occur in the availability of the material and the long-term usage of the cells. Here we describe the isolation, long-term cultivation and characterization of epithelial stem cells (tissue stem cells) from human nasopharynx tissue. The tissue stem cells can be expanded manifold, cryopreserved and reused after thawing. By using the air-liquid interface differentiation method we could achieve organotypic differentiation into nasopharynx-like tissue opening up possibilities for direct infection analyses with pathogenic agents having nasopharynx tissue tropism. A specific interrogation of the tissue stem cell population of the nasopharynx in connection with EBV or HAdV oncogenic mechanisms has not been described thus far. Therefore, we also exploit this primary cell system to try to understand oncogenic disease mechanisms induced by human tumour viruses such as EBV and HAdV.

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STEM CELL NICHES

F-1089

FIVE-COLOR CONFOCAL IMAGING OF HEMATOPOIETIC STEM CELL WITH DIFFERENT NICHE COMPONENTS IN AN OPTICALLY CLEAR MOUSE BONE

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The hematopoietic stem cell (HSC) niche is a specific anatomical location where stem cells reside and receive signals for their maintenance. Recent studies using transgenic mice and high-resolution real time imaging have helped to identify HSC niche components and the molecular signals emanating from them. However simultaneous localization of HSC with its niche has remained a challenge due to use of multiple markers for HSC identification. From our recent transcriptome analysis, we found that endothelial protein C receptor (EPCR+) was predominantly expressed only in long term (LT)-HSC. Flow cytometric analysis further confirmed enrichment of EPCR in LT-HSC with negligible expression in progenitors and differentiated cells. Considering that HSCs are rare and difficult to observe at shallow depths (~100 microns) using traditional whole mount imaging, there is a need for deep imaging in an optically clear bone to understand the localization of HSCs in relation to their micro-environment. Using high resolution confocal imaging coupled with 3D-rendering and computational analysis in an optically clear bone, we demonstrate simultaneous imaging of 5 different colors (1-HSC, 3-niche components and 1-nucleus) with negligible background through the entire depth (300-350 microns) of bone marrow and automated quantitation. Altogether, we simultaneously unravel the quantitative distribution of LT- HSCs along with their niche components in the long bone.

F-1091

AGE- AND REGION-DEPENDENT ARCHITECTURAL REMODELING OF NICHE CONSTITUENTS IN THE HEMATOPOIETIC MICROENVIRONMENT CONTRIBUTES TO HEMATOPOIETIC STEM CELL AGING

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While intrinsic effects of aging in Hematopoietic Stem cells (HSCs) have been explored, less is known about how aging alters the ability of the bone marrow microenvironment (BMME) to support and maintain HSCs. To assess the role of the BMME in HSC aging, we studied aged (20-24 months) compared to young (6-12 weeks) male mice and bone marrow samples from aged (>40 YO) and young (< 40 YO) normal volunteers. In our mouse model we find age-dependent remodeling of marrow cavities with significant expansion in both marrow cavity, and vascular volume in aged animals, which results in redistribution of BMME constituents within two distinct geographic regions including endosteal bone-associated cells (BA) and central marrow (CM) cells. The aged BA cells in aged animals contain fewer phenotypic supportive mesenchymal/osteoblastic progenitors, with a reduction in their CFU activities. This loss of CFU activity is also seen in aged human samples. In the aged murine CM there is significant

expansion of both dysfunctional mesenchymal stem cells, and activated macrophages (MΦ). Expansion of MΦ is also seen in aged human marrows. Ex vivo young HSCs co-cultured with CM cells acquire phenotypic properties of aged HSCs, with increased CD41+ expression. We also find increased expression of the CD41 binding partner CD61 both in vivo in aged HSCs, and on young HSCs co-cultured on primary CM cells. CD41 expression is not seen when CM cells are depleted of CD45+ and Ter119+ cells. In addition sorted aged CM MΦ increased CD41+ young HSCs, suggesting that MΦ can mediate the microenvironmental induction of aging HSC characteristics. Transcriptional analysis of murine CM MΦ identifies an increase in inflammatory signals in aged animals compared to young. Among these signals we identify interleukin-1 beta (IL-1β) and response of interferon gamma (IFN-γ) in aged animals. Addition of IL-1β and/or IFN-γ differentially modulates the immunophenotype of young HSCs in co-culture with young CM. IL-1β increases CD61+ HSCs, while IFN-γ increases CD41+ young HSCs. The combination of both cytokines results in increased expression of both CD41 and CD61 on young HSCs. These data provide evidence that aging differentially impacts and remodels two distinct BMMEs, and that microenvironmental constituents can impart aging characteristics to the HSC pool.

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F-1093

IDENTIFICATION OF DORSAL MSX1+ NEURAL STEM CELLS IN THE ADULT SPINAL CORD NICHE

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ISOLATION OF DORSAL MSX1+ NEURAL STEM CELLS IN THE ADULT SPINAL CORD NICHE Both in human and mice, the central canal region of the adult spinal cord harbors a niche for neural stem cells. These cells represent an attractive cellular source for endogenous repair of spinal cord lesions due to traumatic injury or neurodegenerative diseases such as ALS. This central canal region is heterogeneous and composed of several cell types. The identity of neural stem cells is still ill-defined and controversial. Here we report on the identification of a cell type exclusively present in the dorsal part of the mouse niche and expressing the Msx1 homeogene. These cells constitute the roof of the central canal, have a radial morphology and coexpress FoxJ1, Sox2, Sox9 and GFAP. In the brain, neural stem cell express VEGFR3 (Calvo et al, Genes Dev. 2011 Apr 15;25(8):831-44) and using Msx1-Tomato x VEGFR3-YFP mice, we found that Msx1+ and VEGFR3+ cells largely co-localized. Subset of Msx1+ cells also express VCAM1, a recent marker for neural stem cells. In vitro, Msx1+ cells can generate multipotent neurospheres which can be passaged at least 9 times. They also express high levels of two cytokines, namely BMP6 and GDF10, whose expression is also detected in the dorsal part of the niche. In contrast to the dorsal location in the mouse niche, in man, Msx1+ FoxJ1+ cells are found all around the central canal region, illustrating the divergent organization of the spinal cord niches in rodent and primates.

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F-1095

DEFINING TRANSCRIPTION FACTORS THAT SPECIFY SINUSOIDAL ENDOTHELIAL CELLS IN THE HEMATOPOIETIC STEM CELL NICHE

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Marrow transplantation of hematopoietic stem and progenitor cells (HSPCs) is an important therapy, yet our understanding of donor cell engraftment remains incomplete. Although it's known that sinusoidal endothelial cells in the marrow express niche-specific factors, it is unclear what instructs this subset of cells to interact with HSPCs. Using RNA tomography (tomoseq) - a technique that combines cryosectioning with RNA-seq to examine transcriptome-wide gene expression across a spatial dimension - we identified ~300 genes enriched in the zebrafish caudal hematopoietic tissue (CHT), the hematopoietic equivalent of the mammalian fetal liver. By crossreferencing RNA-seq data on isolated endothelial cells, macrophages, neutrophils, and erythrocytes we determined the cell types in which many of these CHTenriched genes were expressed. In situ hybridization confirmed CHT expression for 74 of 92 tested genes, many of which were specifically expressed by endothelial cells in the CHT. This included

selectin-e (sele), a gene known to be expressed by bone marrow endothelial cells where it promotes HSPC homing. We cloned the 5300 bp upstream of sele and fused it to a GFP reporter. When injected into zebrafish embryos this construct recapitulated sele's CHT-specific expression. By crossing this sele:GFP transgene to a pan-endothelial marker (kdrl:mCherry), we were able to FACS sort CHT endothelial cells and compare them to non-CHT endothelial cells and non-vessel cells. ATAC-seq on these sorted populations revealed 6710 peaks of open chromatin unique to CHT endothelial cells. Closer examination of the upstream region of sele itself revealed a 311 bp peak of open chromatin unique to sele:GFP+;kdrl:mCherry+ niche endothelial cells. Cloning of this small enhancer element in front of GFP was sufficient to drive GFP expression in CHT endothelial cells. HOMER analysis of the sequences under the 6710 unique CHT endothelial peaks identified Ets, Sox, Coup-TFII and GATA sites as the most enriched motifs, establishing a transcription factor code that specifies sinuosoidal endothelial cells in the niche. These studies provide insight into the regulation of niche endothelial cell identity and a tool to specifically express genes in the sinusoidal vessels of the niche.

Funding Source: American Cancer Society.

F-1097

CELL-PRODUCED EXTRACELLULAR MATRIX PROVIDES A TISSUE-SPECIFIC NICHE FOR CONTROLLING THE BEHAVIOR OF MESENCHYMAL STEM CELLS DERIVED FROM BONE MARROW AND ADIPOSE TISSUES

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Research has established the acute sensitivity of stem cells to chemical and physical cues in their local environment (extracellular matrix, ECM). Current stem cell culture methods employ a "one size fits all" paradigm, utilizing synthetic substrates, such as tissue culture plastic (TCP), that cannot recapitulate the native stem cell niche. In order to more appropriately study stem cell behavior, more sophisticated culture environments, reproducing the native, tissue-specific stem cell niche, are essential. To address this need, we prepared bone marrow (BM)- and adipose (AD)-ECM, produced ex vivo by stromal cells derived from these tissues. After decellularization, washing, and sterilization, the cell-free ECMs were used as culture substrates in order to evaluate their ability to mimic the BM and AD tissue microenvironments (niches). We assessed the ability of the ECMs to influence BM- and AD- mesenchymal stem cell (MSC) proliferation, cell spreading/morphology, and differentiation relative to standard TCP. It was found that both types of ECMs promoted MSC proliferation compared to TCP, but this effect was more pronounced when the origin of the MSCs (BM or AD) corresponded to the ECM. Studies of cell spreading morphology showed that each ECM uniquely affected MSCs, irrespective of cell origin. Furthermore, BM- and AD-ECM displayed "tissue-specificity" in directing MSC differentiation towards their respective lineage. MSCs maintained on BM-ECM showed significantly increased responsiveness to BMP-2 (osteoblastic induction), while on AD-ECM, these cells demonstrated greater sensitivity to rosiglitazone (adipogenic induction). BM- and AD-ECMs exhibit considerable differences in biochemical composition as well as unique topographical and mechanical properties, such as surface roughness, fiber alignment and storage modulus. The present study provides evidence that BM- and AD-ECM recapitulate specific elements of their native stem cell niche and constitute an appropriate model for developing tissue-specific culture systems for research and tissue engineering. Furthermore, the ability of BM- and AD-ECM to increase the sensitivity of MSCs' response to induction suggests that the use of tissue-specific ECM may enhance the differentiation capacity of adult MSCs in therapeutic applications.

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EYE AND RETINA

F-1099

EXOGENOUS FACTORS INDUCE ROD PHOTORECEPTOR-SPECIFIC PROGENITORS FROM ADULT MOUSE RETINAL STEM CELLS

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Adult retinal stem cell (RSCs) derived from the ciliary epithelium (CE) of mice can give rise to all retinal

cell types. Taurine, retinoic acid and FGF2/heparin (T+RA+FH) added to clonal RSC colonies increases the number of rods to 90% of all progeny; RSC progeny produce 10% rods when in 1%FBS+FH (pan-retinal conditions) and >90% cones when differentiated in Wnt, BMP4 and TGFβ inhibition. Similarly, T+RA increases the number of rods to >90% of all progeny from embryonic E14 retinal progenitor cells in vitro. We hypothesized that T/RA acts on RSC progeny in an instructive, rather than permissive, manner to enrich rod-specific progenitors. RSCs were clonally isolated from the CE of 4-6 week old mice. We used limiting dilutions (< 1 clone / well) of a fluorescent retroviral construct to label individual progenitor clones in vitro. In addition, single cell sorting isolated non-pigmented and pigmented cells, which were then treated with T/RA for 28 d. Retroviral labeling revealed enrichment in rod-only clones between 1%FBS (13%) to T/RA (over 70%), without affecting clone size or overall cell survival. This argues against selective survival of rod progenitors or post-mitotic rods within a clone. In 1%FBS, clones derived from single non-pigmented progenitors were distributed between non-rod and mixed clones, with a minority of rod-only clones (100% Rhodopsin-positive; n=4 of 28 clones). In T+RA, all clones derived from non-pigmented progenitors (n=34) were rod-only clones, while those derived from pigmented progenitors (n=47 of 48) were almost all non-rod clones. Survival rates of non-pigmented cell derived clones were similar in T+RA and 1%FBS. When cells primed in T+RA are exposed to pan-retinal or cone differentiation conditions, a critical period for the instruction of rod-specific and cone-specific progenitors is revealed. When RSC progeny are subjected to pulses of T+RA at progressively later differentiation time points there is decreased bias for rod specification, suggesting an effect on early progenitor cells. Pathway and network analysis using RNAseq highlighted clustering of stem cell-derived and endogenous rods, as well as candidate markers which might enrich for rod-specific progenitors. This is an important step – no markers exist for such a progenitor and literature is divided on their existence in vivo.

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F-1101

IMPLICATIONS OF SUBRETINAL SUSPENSION TRANSPLANTS OF HESC-DERIVED RETINAL PIGMENT EPITHELIAL CELLS IN A LARGE-EYED PRECLINICAL MODEL OF ADVANCED AGE-RELATED MACULAR DEGENERATION

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Human embryonic stem cell (hESC)-derived retinal pigment epithelial (RPE) cells could replace lost tissue in geographic atrophy (GA), the advanced form of dry age-related macular degeneration, but integration efficacy has yet to be explored when transplanted in suspension into a clinically relevant large-eyed model of GA. hESC-RPE were derived in a xeno-free and defined manner using human recombinant LN-521 matrix. Subretinal bleb injection of phosphate-buffered saline (PBS) or sodium iodate (NaIO3) was used to induce outer neuroretinal and RPE damage, and suspensions of hESC-RPE were transplanted to the subretinal space of naive or PBS- / NaIO3-treated rabbits using a transvitreal pars plana technique. Integration of hESC-RPE was monitored by multimodal real-time imaging and by immunohistochemistry. The damage caused by NaIO3 and to some extent PBS faithfully captured several hallmarks of GA, e.g. different degrees of outer neuroretinal degeneration, RPE hyperautofluorescence and focal RPE loss. In non-pretreated naive eyes, hESC-RPE integrated as subretinal monolayers with preserved overlying photoreceptors, yet not in areas with outer neuroretinal degeneration and native RPE loss. When transplanted into eyes with PBS- / NaIO3-induced degeneration, hESC-RPE failed to integrate. In conclusion, we describe a clinically relevant large-eyed GA model using subretinal injection of NaIO3. Suspension transplants of hESC-RPE fail to properly integrate in eyes with GA-like degeneration in a larged-eyed preclinical model, suggesting that subretinal suspension transplants may not repopulate areas with GA-like degeneration but instead the GA border with a conserved subretinal mileu. These findings are relevant for understanding the success or failure of a stem cell-based treatment for GA.

F-1103

ESTABLISHING A CELL SURFACE MARKER-BASED STRATEGY TO EFFICIENTLY ISOLATE TRANSPLANTABLE HESC-RPE CELLS

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Age-related macular degeneration is one of the most important causes of vision loss in the Western world. Recent clinical trials have suggested that transplantation of human embryonic stem cell (hESC) derived retinal pigment epithelial (RPE) cells, could be used to replace the tissue lost in the degenerative form of macular degeneration. However, differentiation protocols still rely on the manual selection and expansion of RPE cells over other unsought cell types that can emerge along the way. Such approach not only impairs the large-scale production of these cells but also prevents their clinical implementation, as it cannot exclude the risk of tumorigenicity derived from the possible presence of undifferentiated cells in the final product. Aiming to overcome such limitations, we sought to identify unique cell surface markers for the hESC-RPE cells. For that purpose, we screened hESCs, neural epithelial cells and hESC-RPE cells against an antibody library recognizing 240 different cell surface markers. From this screen and subsequent validation, we identified a set of unique cell surface markers that were able to discriminate RPE cells from the bulk of cells that emerged after hESC differentiation. We also identified markers which can be used to evaluate the degree of maturation of the hESC-RPE. Combined with our 2D differentiation protocol under xeno-free conditions on biologically relevant substrates, these novel cell surface markers will enable the semi-automatized and large-scale production of hESC-RPE cells while ensuring the required safety and purity degree of the final cell product for its clinical implementation.

F-1107

PURIFICATION OF CONE PHOTORECEPTORS DERIVED FROM MOUSE INDUCED PLURIPOTENT STEM CELLS

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Retinal diseases such as macular degeneration are characterized by irreversible changes in the photoreceptor (PR) and retinal pigment epithelium (RPE) layers. While PR precursors and progenitors give rise to rods and cones after transplantation (e.g. in rodent and porcine recipients), the alignment and number of integrated cells do not yet permit the full restoration of vision. Although published work shows it is relatively easy to generate large numbers of photoreceptor precursors from pluripotent human and mouse cells in 3D culture, the transition to differentiated cones has remained elusive. In this study, we used mouse induced pluripotent stem cells (miPSC) line labeled with s-opsin green fluorescent protein (GFP). Optic cups were generated in-vitro using a three-dimensional approach using Matrigel®. A NOTCH pathway inhibitor (DAPT) was added to the culture from day 12 to day 15 and s-opsin GFP+ cells were isolated by FACS at day 28 of culture. Subsequently, the isolated s-opsin GFP+ cell populations were characterized by RT-PCR and Flow-Cytometry for expression of cone photoreceptor markers. S-opsin GFP+ isolated cells expressed a number of s-opsin cone photoreceptor specific markers including OPN1SW, THRB2, ARR3, GNGT2, CNGB3, CNGA3, GNAT2, pdeC, SALL3 and DLG4. This study provides a strategy for the generation of large numbers of mouse s-opsin cones that can be used for transplantation, as well as the development of high throughput screening (HTS) assays for cone development and survival. This will enable the development of tissue engineering and drug therapies to treat retinal diseases.

Funding Source: Bertarelli Foundation

NEURAL DEVELOPMENT AND REGENERATION

F-1109

INVESTIGATING THE MOLECULAR PATHWAYS UNDERLYING WARBURG MICRO SYNDROME IN STEM CELL DERIVED HUMAN CORTICAL NEURONS

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Warburg micro syndrome is a rare autosomal recessive genetic disorder associated with severe intellectual disability, postnatal microcephaly, agenesis of the corpus callosum, and microphthalmia. Over 40% of the WARBM cases are associated with mutations in RAB3GAP1 (RAB3 GTPase activating protein 1). RAB3GAP1 is the catalytic subunit of the heterodimeric RAB3GAP complex of RAB3 protein. Previous work has reported a role for RAB3GAP1 in synaptic transmission and plasticity. However, in vivo murine models deficient for RAB3GAP1 do not recapitulate the anatomical brain or eye phenotypes associated with human Warburg micro syndrome. We hypothesize that in humans the deleterious effect of RAB3GAP1 mutations might result from species-specific differential functions of RAB3GAP1 and/or its interacting partners. To begin to test this hypothesis, we first used a mass spectrometry approach to identify novel interactors of RAB3GAP1 in human cells. Candidate proteins identified by mass spectroscopy were further analyzed using co-immunoprecipitation approaches in human embryonic stem cell (hESC) derived cortical neurons. We present here the identification and characterization of two novel interacting partners of RAB3GAP1 in hESC derived cortical neurons. Pathway analysis of RAB3GAP1 interacting proteins highlight membrane trafficking, axon formation and neuronal morphogenesis as major biological processes represented. These preliminary studies suggest that in humans RAB3GAP1 might also regulate neurite formation during human brain development.

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F-1111

INVESTIGATING THE MECHANISMS OF A MULTI-STATE MODEL OF WNT SIGNALING

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The WNT signaling pathway plays a critical role in many developmental processes as well as the maintenance of tissue homeostasis in adults. In addition, dysfunction in WNT signaling results in numerous human diseases. Canonical WNT signaling is classically described by the 'two-state' model. This model posits that in the 'off' state in the absence of a WNT ligand, cytoplasmic β-catenin is continuously degraded by the action of the APC/Axin/GSK-3β destruction complex. In the 'on' state in the presence of WNT ligands, this protein destruction complex is disrupted, allowing β-catenin to translocate into the nucleus where it interacts with the DNA-bound TCF/LEF proteins to regulate target gene expression. However, this 'two-state' model does not adequately explain the mechanisms by which WNT signaling can elicit distinct patterns of target gene expression and cell responses at specific signaling thresholds. For example, in the development and patterning of many tissues, the WNT pathway attains different levels of activity through gradients of WNT signaling activity. In turn, the positional information supplied by these WNT signaling gradients produces the appropriate spatial pattern of cellular differentiation. Elucidating the mechanisms of how a graded WNT signal leads to precise changes in transcriptional responses has been difficult because the lack of an in vitro model where WNT signaling molecules cause distinct cellular phenotypes at different concentrations. To that end, we have developed an in vitro human pluripotent stem cell (hPSC)-based model that recapitulates the same in vivo developmental effects of the WNT signaling gradient on the anterior-posterior (A/P) patterning of the neural tube during early development (Stem Cell Reports. 2014 Dec 9;3(6):1015-28). Using this model along with genome-wide expression analysis (RNA-seq) and DNA binding analysis (ChIP-seq), we are uncovering the mechanisms by which specific levels of WNT activity are translated into precise transcriptional responses and cell identities. Overall, the new insights gained from this research will lead to the better understanding of how various WNT pathway activity levels lead to cancer or other pathological conditions.

Funding Source: NIH

F-1113

GENERATION OF EARLY NEUROEPITHELIAL PROGENITORS FROM HUMAN FETAL BRAIN TISSUE FOR BIOMEDICAL APPLICATIONS

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Major advances in cellular reprogramming for modeling neurological diseases have been achieved. The differentiation of neural progenitor cells (NPCs) from iPSCs and the direct conversion of somatic cells into NPCs emerged into a promising strategy to obtain patient-specific progenitors. However, it remains to be elucidated if those NPCs represent the physiological state. Primary human NPCs from fetal brain tissue might serve an alternative cell source for biomedical and comparative studies. Therefore, we assessed a defined medium composition capable to modulate crucial signaling pathways such as SHH and FGF orchestrating early human development. Indeed, specific culture conditions containing compounds and growth factors enabled the formation of proliferating early neuroepithelial progenitor (eNEP) colonies of homogeneous morphology. Primary eNEPs were monoclonally expanded for more than 45 passages carrying a normal karyotype. Characterization by immunofluorescence, flow cytometry and quantitative RT-PCR revealed a distinct NPC profile including SOX1, PAX6, Nestin, SOX2 and CD133. NOTCH and HES5 upregulation combined with non-polarized morphology indicates an early neuroepithelial identity. Interestingly, eNEPs were detected to be of ventral midbrain/hindbrain regional identity. The validation of yielded cell types upon differentiation suggests a strong neurogenic potential, but also astrocytes and putative myelin structures indicating oligodendrocytes were identified. Electrophysiological recordings revealed functionally active neurons and immunofluorescence indicated GABAergic, glutamatergic, dopaminergic and serotonergic subtypes. Additionally, putative physiological synapse formation was observed by immunostainings and ultrastructural examination. Notably, neurons positive for the peripheral neuronal marker Peripherin could be found suggesting the potential of eNEPs to give rise to cells of neural tube and neural crest origin. Taken together, the presented study demonstrates the derivation of novel fetal-derived eNEPs which might help to elucidate mechanisms of early human neurodevelopment and serve as a comparative cell line. Moreover, eNEPs potentially represent a novel source for cell replacement, drug screening and neural tissue engineering.

F-1115

THE TRANSCRIPTION FACTOR GLIS3 DIRECTLY REGULATES WNT SIGNALING TO SPECIFY REGIONAL IDENTITY TOWARD HPSC-DERIVED NEURAL PROGENITOR CELLS

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Human pluripotent stem cells (hPSCs), which differentiate into neural progenitor cells (NPCs) and subsequently into functional neuronal subtypes, provide great tools for investigating and understanding functional neuronal subtypes and CNS development. Neuronal induction occurs by default in the primitive ectoderm, forming anterior neural tissue, and thereafter a number of factors can posteriorize this anterior neuroectoderm. However, our knowledge of the mechanisms that control the initial anterior-posterior (A/P) patterning of neuronal development is limited. Here we show that Kruppel-like zinc finger transcription factor Gli-similar 3 (Glis3) can control the A/P patterning by regulating endogenous WNT signaling during neuronal induction. Glis3 expression causes a shift in NPC differentiation pathways from the default anterior NPCs (aNPCs) pathway to the posterior NPCs (pNPCs) pathway. This shift in regional identity of hPSC-derived NPCs was shown to be related to an induction of WNT3a expression. Data showing that WNT inhibitors (Wnt-C59 and IWP2) greatly abrogated Glis3-induced posterior NPC differentiation support the conclusion that Glis3 promotes the differentiation of hPSCs into posterior NPCs via the induction of WNT3a expression and repression of WNT inhibitors. Genome-wide occupancy and transcriptomic analysis demonstrated that Glis3 regulates WNT gene expression directly by binding to Glis binding sites in the regulatory regions of several WNT genes. Our study identifies Glis3 as a critical regulator of anterior-posterior neural lineage determination by regulating WNT signaling and WNT gene transcription. In addition, it provides a molecular mechanism of Glis3 by which hPSCs specify their regional identity for the neuronal lineage, improving their scientific and therapeutic utility in the human nervous system lineage.

F-1117

DIFFERENTIATION OF INFLAMMATION-RESPONSIVE ASTROCYTES FROM GLIAL PROGENITORS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Astrocyte dysfunction and neuroinflammation are detrimental features of multiple central nervous system pathologies. Therefore, methods for generating functional and inflammation-responsive human astrocytes represent a valuable advance in the study of neuroinflammation and related CNS disorders. Here we report an efficient method for generating astrocytes from human induced pluripotent stem cells (iPS) via an intermediate glial progenitor cell (GPC) stage. The generated astrocytes showed levels of glutamate uptake and calcium transients comparable to those observed in primary human astrocytes. Notably, astrocytes derived by this method are inflammation responsive, as stimulation of iPS-derived astrocytes with IL-1β or TNFα elicited a strong and rapid pro-inflammatory response. RNA-sequencing analysis of stimulated iPS-derived astrocytes revealed a transcriptomic signature consistent with the activation of primary human astrocytes with key inflammation-related genes upregulated. Further, IL-6 and IL-8 cytokines were detected in a large proportion of iPS-derived astrocytes following stimulation. Here we demonstrate that astrocytes can be generated efficiently from human iPS-derived GPCs, and that these functional and inflammation-responsive astrocytes may be utilized as a novel tool for studying components of neuroinflammation in vitro.

F-1119

CRANIAL BONE DERIVED MESENCHYMAL STEM CELLS CULTURED IN SIMULATED MICROGRAVITY HAVE HIGHLY NEUROPROTECTIVE EFFECT AGAINST STRESS INDUCED NG108-15 CELL DEATH IN VITRO

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Central nervous system disease causes from primary damage (initial mechanical damage) and secondary damage, which is not able to achieve radical cure. Previous studies have suggested that cell-based therapy using mesenchymal stem cells (MSCs) have possible therapeutic effects. We have reported that MSCs cultured in simulated microgravity (MG) had highly therapeutic potential in spinal cord injury model. Recently, it has been proposed and commonly accepted that the functional benefits of MSCs transplantation are due to a neuroprotective effect. However, the neuroprotective effect of MSCs cultured in MG is still unclear. Therefore, the present study aimed to determine the neuroprotective effect of MSCs cultured in MG against secondary damage model in vitro. Cranial bone derived mesenchymal stem cells (cMSCs) were isolated from human cranial bone marrow samples collected from front-temporal cranial bone waste during neurosurgical procedures. cMSCs were cultured in normal ground (1G) or MG environment using Gravite®. After 5 days culture, the mRNA expression of cMSCs was analyzed and the culture medium were collected from both culture conditions as conditioned medium (CM). NG108-15 cells exposed to inflammatory or oxidative stress were used as a secondary damage model to evaluate the neuroprotective effect of CM. The NG108-15 cells were cultured in 1G-CM, MG-CM, or fresh cMSCs growth medium (Ctrl) (with 200 ng/ml Lipopolysaccharide or 500 μM H2O2). The cells were collected 24 h after exposure to stress for analyzing survival rate or mRNA expression. cMSCs cultured in MG showed significantly higher expression of hepatocyte growth factor (HGF) compared with those cultured in 1G. The survival rate of NG108-15 cells exposed to inflammatory or oxidative stress was significantly higher in cells cultured in MG-CM than those cultured in 1G-CM or Ctrl. As a results of mRNA expression, Tnfa expression level in the inflammatory stress exposed NG108-15 cells was significantly lower in

cells cultured in MG-CM than those cultured in Ctrl. The Bax/Bcl2 ratio in the oxidative stress exposed NG108-15 cells was significantly lower in cells cultured in MG-CM than those cultured in Ctrl. These results suggested that Gravite® culture method is useful strategy to increase the neuroprotective effect of cMSCs.

F-1121

D-SERINE CONTROLS THE GROWTH AND SELF-RENEWAL OF MOUSE HIPPOCAMPAL NEURAL STEM/PROGENITOR CELLS

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During neural development, neural stem/progenitor cells (NSPCs) generate neurons, oligodendrocytes and astrocytes in specific temporal orders, while maintaining undifferentiated cell populations. However, how NSPCs self-renew/maintain undifferentiated cell populations remain still unclear. Accumulating evidence suggest that NSPCs are pharmacologically and/or electrically able to respond to neural activities and change their differentiating properties. Here, we show that D-serine, a regulator of NMDA receptor signaling, plays crucial roles not only in time specific neuro-gliogenesis, but also in the maintenance/self-renewal of NSPCs. D-serine, which is generated from L-serine by serine racemase (SR), starts to act at postnatal day 7 (P7) when SR starts to express in the developing nervous system of mouse. Ca imaging revealed that P8 SR+/- derived NSPCs responded to NMDA administration while the response was down-regulated in SR-/-, suggesting that NMDA receptors are functionally expressed in SR+/- NSPCs and that the activity of NMDA receptors is regulated by SR expression. We then found that P8 SR-/- multi-potent NSPCs were biased to generate neurons and oligodendrocytes rather than astrocytes compared with wild type (WT)/SR+/- NSPCs. Clone assay of NSPCs revealed that SR+/- NSPCs were mainly astrogenic and lineage-restricted astrocyte progenitors, while SR-/- NSPCs were mainly neurogenic and oligodendrogenic. The phenotype in SR-/- NSPCs was rescued by administration of D-serine, suggesting that D-serine/SR are involved in the control of neural fate specification. We also found that an increase in the number of NSPC clones while decrease in the size of clones during the growth of the SR-/- derived NSPC populations. D-serine administration reduced the number of clones while enhanced the growth of clones, supporting an idea where SR/D-serine negatively regulate self-renewal of NSPCs while enhancing the growth of the NSPCs. Thus, D-serine/SR controls both sequential specification of neural cells and the maintenance/self-renewal of the hippocampal NSPCs.

F-1123

SIMULATED MICROGRAVITY CULTURE INCREASES THE NEUROPROTECTIVE POTENTIAL OF HUMAN CRANIAL BONE DERIVED MESENCHYMAL STEM CELLS IN BRAIN INJURY MODEL

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Mesenchymal stem cells (MSCs) have been proposed as a highly useful candidate as grafting cells in cell-based therapy. Previous study suggested that stem cells derived from different tissues have distinct characteristics. Therefore, we need to choose the optimal MSCs for treatment depending on patient's diagnosis or symptoms. Recently, we reported that MSCs derived from cranial bone marrow (cMSCs) have high neurogenic potential compared with MSCs derived from iliac bone marrow. Moreover, we also reported that MSCs cultured in simulated microgravity (MG) had highly therapeutic potential in brain or spinal cord injury model. However, the therapeutic effect of cMSCs cultured in simulated microgravity is still unclear. Therefore, the present study aimed to determine whether simulated microgravity increase the neuroprotective effect of cMSCs. cMSCs were isolated from human cranial bone marrow samples collected from fronto-temporal cranial bone waste during neurosurgical procedures. Cryogenic brain injury model mice were used in this study. Model mice were divided into three groups, transplantation of cMSCs cultured in normal ground (1G) (group 1G), transplantation of cMSCs cultured in MG (group MG) using the Gravity Controller "Gravite®", and only PBS administration (group Ctrl). MSCs were transplanted intravenously 24 h after brain injury. The mRNA expression of cMSCs before transplantation and brain injury lesion area was analyzed using real-time PCR. cMSCs cultured in MG showed significantly higher expression of hepatocyte growth factor (HGF) and transforming growth factor (TGFb) compared with those cultured in 1G. In the results of animal experiment, mice in group MG demonstrated significant functional improvement compared with mice in group 1G or group Ctrl. The mRNA expression of tumor necrosis factor alpha (Tnfa) and Bax/Bcl2 was significantly lower in brain lesion area in group MG than those in group Ctrl. These results suggested that simulated microgravity

culture is useful strategy to increase the neuroprotective effect of cMSCs using cell-based therapy.

F-1125

PRECLINICAL CHARACTERIZATION OF FROZEN-THAWED DOPAMINERGIC CELL PREPARATIONS FOR PARKINSON'S DISEASE AUTOLOGOUS CELL REPLACEMENT THERAPY

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There are approximately 1.5 million diagnosed cases of Parkinson's disease (PD) in the U.S. Patients with this chronic progressive disorder present with motor symptoms characterized by tremor, bradykinesia, rigidity and postural instability. At the onset of symptoms and diagnosis ~ 70% of the midbrain DA neurons have already degenerated. L-DOPA can initially restore dopamine (DA) levels and motor function, but with time the therapeutic window becomes increasingly narrow with L-DOPA induced dyskinesia as a common side effect. Although deep-brain-stimulation (DBS) also can alleviate motor symptoms, such interventions ultimately lead to repeat procedures, limitations for patients in receiving other medical procedures and high medical costs. Cell replacement therapy has proven beneficial in clinical studies using cell preparations derived from fetal ventral midbrain. While fetal cell transplantations are not scalable for a larger patient population and require immunosuppression, induced pluripotent stem cells (iPSCs) are a promising alternative. iPSCs generated from affected PD patients can be differentiated into midbrain dopaminergic cells and used for autologous transplantation. The proof-of-concept of autologous transplantations using differentiated iPSCs has previously been shown by us in non-human primates. In current pre-clinical efforts, we have generated iPSCs from human PBMCs using episomal reprogramming and xeno-free derivation conditions. The iPSCs have been differentiated into midbrain DA neurons using a xeno-free differentiation protocol and the differentiated cell preparations frozen and thawed with good viability and reliable reproducibility. The frozen-thawed cell preparations have been characterized based on cell viability and stability, cell content and reproducibility of differentiated cell batches, and in vivo functionality after xeno transplantations into rodents. These data provide strong support for the clinical translation of iPSC-derived midbrain DA neuron cell therapy.

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F-1127

PURIFICATION OF MOUSE CORTICAL NEURONS USING L1CAM

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Over the last few years, many reports have shown that pluripotent stem cells (PSCs) are useful tools for cell replacement therapy. On the other hand, regeneration of mature central nervous system (CNS) in response to cell loss is limited because of the complexity. In the cortex, pyramidal neurons project their axons to the target area far from the cortex and perform input and output of the neural network there. To facilitate point-to-point reinnervation of target areas by cell transplantation, we focus on purifying cortical neurons from mouse frontal cortex. In order to accomplish this, we first generated CTIP2, a corticospinal motor neuron marker, knock-in mouse embryonic stem cells (mESCs). Mouse ESC-derived cortical neurons such as FOXG1+, TBR1+, BRN2+ cells were observed in vitro. To identify a cell surface marker of cortical neurons, then we compared gene expression profiles between mESC-derived CTIP2::GFP+ cells vs. mESC-derived CTIP2::GFP- cells. Among these screening, L1cam was identified as up-regulated gene in the CTIP2::GFP+ cell population. L1cam was expressed from intermediate zone to cortical plate during mouse development. When we performed cell sorting of E14.5 mouse frontal cortex using anti-L1CAM antibody, CTIP2+ cells were more frequently observed in L1CAM+ population compared with unsorted and L1CAM- populations (75.8% vs. 36.4% vs. 13.9%). Moreover, mRNA levels of other cortical plate cells such as Foxg1+ and Tbr1+ were higher in L1CAM+ population than unsorted or L1CAM- populations. On the other hand, mRNA level of ventricular zone cells such as Pax6+ was lower in L1CAM+ population than unsorted or L1CAM- populations. Finally, E14.5 EGFP mouse frontal cortex-derived sorted cells were transplanted into the brain of neonate mouse. 2 months later, L1CAM+ graft-derived fibers were more frequently observed in the corticospinal tract and the corticothalamic tract. In conclusion, a cell surface marker for cortical neurons, L1CAM, can provide a tool for regeneration of cortical circuits.

NEURAL DISEASE AND DEGENERATION

F-1131

A FLEXIBLE IPSC-NEURONAL PLATFORM FOR PATHOGENESIS AND DRUG DISCOVERY RESEARCH

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Infection of neurotropic viruses can cause structural and functional changes of the CNS, leading to long-term neurological sequelae. An improved understanding of the pathogenesis of neurotropic viruses is important to develop efficacious antiviral interventions, but the investigation of CNS infections in humans has been hampered by the absence of satisfactory human neuronal cell models. Similarly, drug discovery for CNS infections has been hindered by lack of human cellular platforms suitable for high-throughput drug screening. Induced pluripotent stem cells (iPSCs) can potentially provide a solution to these hurdles. We have developed an efficient, cost-effective method to generate variable numbers of neuronal cells on 6-, 96- or 384-well plates, composed primarily of cells with glutamatergic features. We have used this platform to model aspects of the neuropathogenesis of human cytomegalovirus (HCMV), herpes simplex virus, type 1 (HSV-1) and zika virus. For the first time, we have modeled HSV-1 latent infection in cortical neurons and showed that the latent form of the virus, which is considered innocuous, alters the expression of cognition-relevant glutamate receptors and ion channel genes. Our iPSC-based cellular platform has also enabled us to identify novel classes of small molecules exhibiting potent anti-herpetic activity. We are currently validating the antiviral activity of these novel inhibitors using scaffold-free three-dimensional (3D) neuronal cultures generated in 96-well plates.

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F-1133

INVESTIGATING THE ROLE OF ABCC9 IN HIPPOCAMPAL SCLEROSIS AND ALZHEIMER'S DISEASE

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Hippocampal Sclerosis (HS) is a neuropathological ailment in the hippocampus consisting of gliosis and extensive neuronal cell damage, and is also widely identified in Alzheimer's disease (AD) patients. Therefore it is important to investigate the underlying mechanisms of HS development. AD pathology is depicted by the progression of intracellular neurofibrillary tangles (NFTs), extracellular amyloid plaques (Aβ plaques), the death of neurons and the loss of synapses. However, the genomic basis has yet to be discovered. Recently, genome-wide association studies (GWAS) among HS patients suggest that ATP-binding cassette sub family C member 9 (ABCC9), a potassium channel subunit, is associated with HS-aging pathology. My goal is to investigate the molecular basis of ABCC9 in HS and AD development. To do that, I propose to generate genetic variants of ABCC9 in human induced pluripotent stem cells (hiPSC) and test the functional impact in hiPSC-derived neurons. I will use hiPSC and genome-editing technology (CRISPR/CAS9 system) to create HS associated SNPs within ABCC9. Specifically, two risk SNPs have been identified which are in linkage disequilibrium. ABCC9 rs704180 has been associated with multifocal atrophy and rs704178, has shown positive involvement with HS pathology. The risk allele for rs704178-G is linked to rs704180-A. I will generate hiPSCs carrying the following variants: homozygous rs704178-G and rs704180-A, rs704178-C and rs704180-G, and heterozygous rs704178-G/C and rs704180-A/G. The hiPSCs will be differentiated into brain neurons. Using these neurons, I will conduct various tests to examine phosphorylated Tau protein levels, Aβ level, and APP processing. Currently, I have been conducting transfections on JCVB iPS using CRIPSR/Cas9 system and donor oligos with the altered SNP sequences. I will examine changes of ABCC9 mRNA level using qPCR and of ABCC9 protein level using Western blotting analysis. Following this, trafficking patterns and levels of Aβ and the change of phosphorylated Tau will be examined. This work will shed light on the molecular mechanism of ABCC9 in the development of HS and AD. This may provide new insight on the neurodegenerative progress of dementia in patients.

F-1135

DIFFERENTIATION OF DOPAMINERGIC NEURAL CELLS FROM HUMAN ADIPOSE-DERIVED STEM CELLS

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Parkinson disease (PD) is a neurodegenerative disease that is attributed to a progressive loss of dopaminergic neurons within the substantia nigra. The current therapy for PD mostly relies on symptomatic treatments; therefore, it is desirable that regenerative medicine strategies will be developed for PD. Recent progress in stem cell research has raised hope for the development of stem cell therapies, which can be a valuable tool in regenerative medicine. Human adipose tissue–derived stem cells (hADSCs) are multipotent stem cells that can differentiate into various types of cells, including neuronal cells. Moreover, because of their reduced risk of tumorigenesis, and their hypoimmunogenicity and immunomodulatory effects, hADSCs are an attractive material for cell therapy and tissue engineering. In this study, we attempted to develop a dopaminergic neuronal differentiation strategy from hADSCs. We investigated a dopaminergic neuronal differentiation potential of hADSCs by introducing four transcription factors, Achaete-scute homolog 1(ASCL1), forkhead box protein A2 (Foxa2), LIM homeobox transcription factor 1 alpha (Lmx1a), and Nuclear receptor related 1 (Nurr1). Intriguingly, addition of basic fibroblast growth factor (bFGF) caused a formation of spheroid like cells when introducing Foxa2 or Lmx1a into hADSCs. Nestin expression was observed to be upregulated in these cells, suggesting that these spheroid-like cells were neuronal stem cells. Further induction of neuronal differentiation resulted in the increased expression of Tyrosine hydroxylase (TH), Neurofilament-M (NF-M), and Microtuble-associated protein 2 (MAP2) in these cells. In addition, enzyme-linked immunosorbent assay revealed that dopamine secretion was significantly increased in hADSCs introduced with Foxa2or Lmx1a. These data suggest that the hADSCs introduced with Foxa2 or Lmx1a have the possibility to differentiate into dopaminergic cells via neural stem-like cells. We also try to differentiate into dopaminergic cells from iPSCs derived from hADSCs, and found that they efficiently differentiated into dopaminergic neurons. Our study thus may help developing effective therapy for PD.

F-1139

EXPLORING THE CONNECTION BETWEEN ER STRESS AND NEURON EXCITABILITY IN IPSC-DERIVED ALS MOTOR NEURONS

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ALS is a fatal neurodegenerative disease characterized by progressive loss of motor neurons. Multiple potential underlying pathological processes have been identified, including ER stress and hyperexcitability. However, whether neuronal activity affects cellular stress or cellular stress has an impact on neuronal activity, and whether they each or both contribute to neural degeneration is still not clear. Using iPSC differentiated motor neurons from an ALS patient carrying the SOD1(A4V) mutation, we find that the mutant neurons are hyperexcitable compared to neurons from a line where the mutation was corrected, by both multiple electrode array (MEA) recording and GCaMP imaging. Mutant neurons are more vulnerable to excitotoxicity, suggesting that neuronal hyperexcitability may be pathogenic. To monitor cellular ER stress levels, we developed an XBP1-IRE reporter system suitable for high content imaging. XBP1 is a transcription factor that responds to ER stress and mediates the up-regulation of unfolded protein response genes. Utilizing this reporter we observed increased XBP1-splicing activity in ALS motor neurons, suggesting enhanced basal ER stress levels. We also found that ALS motor neurons are more vulnerable to ER stressor induced cell death. Coexpression of the XBP1-IRE and GCaMP reporter in mutant and control motor neurons enables us to measure both ER stress and excitability in individual neurons and explore whether neuronal activity, ER stress and cell death are linked in ALS.

F-1141

ADIPOSE-DERIVED STEM CELLS STIMULATED WITH N-BUTYLIDENEPHTHALIDE EXHIBIT THERAPEUTIC EFFECTS IN A MOUSE MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) causes motor dysfunction and dopaminergic cell death. Drug treatments can effectively reduce symptoms, but often cause unwanted side effects. Stem cell therapies using cell replacement

or indirect beneficial secretomes have recently emerged as potential therapeutic strategies. Although various types of stem cells have been proposed as possible candidates, adipose-derived stem cells (ADSCs) are easily obtainable, more abundant, less ethically disputed, and able to differentiate into multiple cell lineages. However, treatment of PD using adult stem cells is known to be less efficacious than neuron or embryonic stem cell transplantation. Therefore, improved therapies are urgently needed. n-Butylidenephthalide (BP), which is extracted from Angelica sinensis, has been shown to have anti-inflammatory and neuroprotective effects. Indeed, we previously demonstrated that BP treatment of ADSCs enhances the expression of neurogenesis and homing factors, such as nuclear receptor related-1 protein, stromal-derived factor 1, and brain-derived neurotrophic factor. In the present study, we examined the ability of BP-pretreated ADSC transplantation to improve PD motor symptoms and protect dopamine neurons in a mouse model of PD. We evaluated the results using neuronal behavior tests, such as beam walking, rotarod, and locomotor activity tests. ADSCs with or without BP pretreatment were transplanted into the striatum. Our findings demonstrated that ADSC transplantation improved motor abilities with varied efficacies and that BP stimulation improved the therapeutic effects of transplantation. Dopaminergic cell numbers returned to normal in ADSC-transplanted mice after 22 days. In summary, stimulating ADSCs with BP improved PD recovery efficiency. Thus, our results provide important new strategies to improve stem cell therapies for neurodegenerative diseases in future studies.

F-1143

NEUROPROTECTIVE EFFECTS OF HUMAN WHARTON'S JELLY MESENCHYMAL STEM CELLS IN AN OXIDATIVE STRESS MODEL IN RAT NEURAL CELLS

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Oxidative stress is a factor involved in the pathogenesis of many human diseases. In the central nervous system specifically, it is a key mediator of cell damage in neurodegenerative diseases including Alzheimer's Disease, Parkinson Disease and Amyotrophic Lateral Sclerosis, and it's modulation could ameliorate the progression of these pathologies. The use of Mesenchymal Stem Cells (MSC) has become a promising treatment option in cell therapy due to their regenerative potential. Indeed, it has been shown that MSC have immunomodulatory and anti-inflammatory properties mediated by paracrine mechanisms, and their beneficial effects on animal models of the neurodegenerative diseases above mentioned have already been proved. More recent studies have shown that the administration of extracellular vesicles (EVs) secreted by MSC could be a new form of cell-free therapy. Besides, MSC are characteristic for being highly resistant to increased levels of reactive oxygen species, since they have tightly controlled mechanisms to regulate oxidative stress. Here, we tested the direct effect of MSC from human Wharton's Jelly (WJ-MSC) and their EVs in neural cells exposed to oxidative stress. To this aim, rat hippocampal neural cells were co-cultured with WJ-MSC using transmwell membranes or treated with WJ-MSC EVs during 24h and incubated with hydrogen peroxide (H2O2). Hydrogen peroxide caused an increase in intracellular reactive oxygen species in neurons, which was significantly reduced when neural cells where previously co-cultured with WJ-MSC or treated with EVs. The viability of neural cells was also reduced 24h after exposure to hydrogen peroxide. Maintaining the co-culture with WJ-MSC during the 24h following H2O2 exposure produced a significant increase in cell viability. Treatment with EVs did not produce significant changes in neural cells viability after H2O2 exposure at the doses used. Finally, we tested if the WJ-MSC adhered to the transwell membranes during the co-culture were resistant to oxidative stress. Indeed, it was shown that the viability of these cells was not altered by exposure to H2O2. This work shows that WJ-MSC have an antioxidant and neuroprotective potential that can be further investigated for the treatment of neurodegenerative diseases.

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F-1145

SCREENING OF COMPOUNDS PROMOTING MATURATION AND AGEING IN IPSC-DERIVED NEURONS

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Induced pluripotent stem cells (iPSC) are useful as models of neurodegenerative disease such as Parkinson's disease (PD) and Alzheimer disease. However, iPSC-derived neurons require long-term

cultivation for their maturation and exhibition of disease-specific phenotypes of late onset neurodegenerative disorders. In this study, we screened compounds that promote maturation and differentiation of iPSC-derived neurons. We used a lentiviral synapsin-GFP reporter as an indicator of neuronal maturation of iPSC-derived neurons. We screened 400 inhibitor chemicals and found that several compounds accelerated differentiation and maturation of iPSC-derived neurons. During dopaminergic neuron induction from iPSC via neuroshere, these compound significantly increased the number of DsRed-synapsin positive mature neurons and decreased number of Sox2 positive neural progenitors. We next investigated whether these compounds accelerated neurodegenerative phenotypes in iPSC-derived neurons of PD patients. Several compounds that related Atm kinase also promoted neurite degeneration in PD-iPSC derived dopaminergic neuron. These phenotypes were observed significantly earlier (15~20days) compared to the conventional method (~about 30~50 days). We are now investigating the molecular mechanisms of these maturation and aging effects induced by these chemicals.

F-1147

INTRAVENOUS INJECTION WITH DEDIFFERENTIATED FAT CELLS AMELIORATED NEONATAL HYPOXIC ISCHEMIC BRAIN INJURY, POSSIBLY VIA PARACRINE EFFECTS

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Neonatal hypoxic ischemic encephalopathy (HIE) remains a major cause of mortality and persistent neurological disabilities in affected individuals. Dedifferentiated fat (DFAT) cells are derived from mature adipocytes via a dedifferentiation strategy called ceiling culture. Their abundance and readily availability might make them an ideal therapeutic tool for the treatment of HIE. In this study, we aimed to determine whether the outcome of HIE can be improved by DFAT cell treatment. Hypoxic ischemic (HI) injury was achieved by ligating the left common carotid artery in 7-day-old rat pups, followed by 1 h exposure to 8% O2. At 24 h after hypoxia, DFAT cells were injected at 105 cells/pup into the right external jugular vein. Animals were sacrificed 48 h after the insult and sections of the brain were stained to assess several acute injury markers. From the 24th day of age, the behavioral tests were performed with the rotarod treadmill, cylinder test, and the novel object recognition (NOR) test. In vitro conditioned medium (CM) prepared from cultured DFAT cells was added to neuronal cell cultures, which were then exposed to oxygen-glucose deprivation (OGD). The number of cells that stained positive for the apoptosis marker, active caspase-3, decreased by 73% and 52% in the hippocampus and temporal cortex areas of the brain, respectively, in the DFAT-treated pups. Similarly, the numbers of ED-1-positive cells (activated microglia) decreased by 66% and 44%, respectively, in the same areas in the DFAT-treated group. The number of cells positive for the oxidative stress marker, 4-hydroxyl-2-nonenal, decreased by 68% and 50% in the hippocampus and the parietal cortex areas, respectively, in the DFAT-treated group. The hypoxic ischemic insult led to a motor deficit, where it significantly affected the vehicle group, whereas no difference was confirmed between the DFAT and sham groups. However, the NOR test indicated no significant differences between any of the groups. According to in vitro experiments, the cell death rates in the DFAT-CM treated cells were significantly lower than those in the controls when DFAT-CM was added 24 to 48 h prior to OGD. Our results indicate that intravenous injection with DFAT cells is effective for ameliorating neonatal HI brain injury, possibly via paracrine effects.

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F-1149

THE PROINFLAMATORY EFFECT OF HUMAN BONE MESENCHYMAL STEM CELLS IN T-LYMPHOCYTES OF PATIENTS WITH MULTIPLE SCLEROSIS

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Contradictory results have been reported on the immunomodulatory efficacy of Mesenchymal Stem Cells (MSCs) in different autoimmune diseases. Therefore, it was determined whether there is a change

in the proliferation and modification of the cytosine secretion pattern in the T lymphocyte response (LT) of patients with Multiple Sclerosis (MS) under different co-culture stimulations with allogeneic MSCs. MSCs in two 1: 1 and 1:10 ratios were co-cultured with mononuclear cells (MNC) from MS patients to determine their immunomodulatory effect. Measurement of proliferation by carboxyfluorescein diacetate succinimidyl ester (CFSE) and TH1 / TH2 soluble cytokine profile was performed by CBA (Cytometric Bead Array). Anti-CD3 / CD28 stimulation of LT increased proliferation (p = 0.026) in co-culture, from an average of 26% (RI: 13.32% -34.7%) in the absence of MSC at 64.95 % With MSC1: 1 (IR: 31.25% -90.22%). Similar to the proliferation of LT with stimulated myelin 15ug / ml (p = 0.002), step from 0% (RI: 0% -0.1%) to 0.2% (RI: 0.1% -2, 17%) in the presence of 1: 1 MSC. An increase in IL-6 concentration was observed in the presence of MSC, which increased 855-fold under the myelin stimulus. A modified pattern of inflammatory cytosine secretion was observed in the presence of MSC, in contrast with the results of inhibition of dose dependent proliferation. But consistent with results showing that MSCs may have an immunomodulatory or pro-inflammatory profile.

F-1151

FUNCTIONAL ASSAYS TO ACCESS NEUROTOXICITY WITH HUMAN IPSC-DERIVED NEURONS

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Human cell types differentiated from induced pluripotent stem cells (iPSC) offer a unique source of cellular material for toxicity screening. For example, several studies have been presented on the use of iPSC-derived cardiomyocytes and hepatocytes in safety toxicology investigations. Equally important is comparative neurotoxicity assessment in neuronal cell types for safety toxicology and uncovering molecular mechanisms underlying excitotoxic cell death pathways. Advances in iPSC technology provide access to previously unattainable cell types from the human brain opening new opportunities to address the shortcomings and limitations of rodent primary cells and immortalized cell lines. Here we present the neurotoxic effects of the excitatory neurotransmitter glutamate and related compounds across a panel of cell types, including iPSC-derived GABAergic cortical neurons and glutamatergic cortical neurons, as well as midbrain dopaminergic neurons. For comparison, the cytotoxicity of a broad spectrum kinase inhibitor, staurosporine (STS), was also evaluated in parallel. To achieve robust signals across the three iPSC-derived neuronal subtypes, we have optimized the cell culture protocols (i.e., media, time in culture, cell plating density, etc.). Under the various conditions tested, we observed differential responses to glutamatergic compounds (e.g. glutamate, NMDA, AMPA, and kainic acid) versus STS. Importantly, toxicity induced by glutamate could be reversed with antagonists of the AMPA and NMDA receptors, DNQX and D-AP5, respectively, suggesting the toxicity responses were mediated by a pathway originating from neuronal synaptic receptors. . We also provide measurements of electrical activities of iPSC-derived neurons on multi-electrode arrays (MEA) to assess the effects of both developmental and environmental neurotoxicants. Overall, these iPSC-derived neurons exhibit functional glutamate pathways that respond appropriately to known agonists and antagonists, thus providing biologically relevant models for identifying emerging targets for excitotoxicity research. Together with the developmental and environmental toxicity studies, these data establish a clear utility for iPSC-derived neurons in toxicology studies.

F-1153

CELL LINE RESOURCES FROM PARKINSON'S PROGRESSION MARKERS INITIATIVE (PPMI) PARTICIPANTS

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The Parkinson's Progression Markers Initiative (PPMI) is a longitudinal observational study conducted at over thirty sites that collects data and biospecimens from Parkinson's patients, controls, and participants with risk factors for Parkinson's disease (PD), such as genetic mutations, hyposmia, and REM Sleep Behavior Disorder, for up to five years. PPMI makes these data and biospecimens rapidly available to qualified investigators to enable biomarker research. In addition to blood, nucleic acids, urine, and cerebrospinal fluid (CSF), PPMI is also committed to obtaining and distributing a range of cell lines, including uniformly collected fibroblasts and induced pluripotent stem cells (iPSCs), from these well-characterized participants to be used for biomarker research, therapeutic development, drug screening, and disease modeling. PPMI includes fibroblast and iPSC collections as part of two ancillary studies. The first sub-study, performed in collaboration with the New York Stem Cell Foundation (NYSCF), derived fibroblasts and iPSCs from skin biopsies. Fibroblasts

and iPSCs from twenty idiopathic PD patients and five controls from one site are currently available for request from this ancillary study. In order to provide cell lines to as many qualified researchers as possible, PPMI is prioritizing the expansion and characterization of these iPSC and fibroblast resources by WiCell and the Rutgers University Cell and DNA Repository (RUCDR), respectively. The second ancillary study is currently being conducted in collaboration with Cellular Dynamics International (CDI). This ancillary study uses a blood-based collection protocol and is being carried out at ten sites. The sites participating in this ancillary study aim to have 135 collections from PD patients, controls, prodromal participants, and affected and unaffected carriers of genetic mutations associated with PD by mid-2017. The iPSCs generated in collaboration with CDI are made available on a rolling basis as they complete reprogramming. All PPMI cell lines are housed at the biorepository at Indiana University. All applications for PPMI cell lines are reviewed by the Biospecimen Review Committee. To apply for access to PPMI cell lines, please visit http://www.ppmi-info.org/access-data-specimens/request-cell-lines/.

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F-1155

FUNCTIONAL BRAIN REPAIR THROUGH IN VIVO CELL CONVERSION

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Brain injury such as stroke not only causes neuronal loss but also activates glial cells. We have recently developed an innovative in vivo cell conversion technology to directly convert reactive glial cells into functional neurons inside the mouse brain (Guo et al., Cell Stem Cell, 2014, selected as BEST of 2014 article). This is achieved through in vivo expression of a single neural transcription factor NeuroD1 in the reactive astrocytes in injured mouse brain or Alzheimer's disease mouse model. Our in vivo cell conversion technology makes use of internal glial cells to regenerate new neurons, making it possible for the first time in history to reverse glial scar back to neural tissue. Such internal cell conversion method will avoid cell transplantation and immunorejection. We have further discovered a cocktail of small molecules that can directly convert cultured human astrocytes into functional neurons (Zhang et al., Cell Stem Cell, 2015), paving the way for a potential drug therapy for human brain repair. Our most recent data demonstrate a significant neural repairing effect of NeuroD1 in stroke animals including mice, rats, and non-human primates, laying a foundation toward future clinical trials.

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CANCERS

F-1159

ONCOGENE EXPRESSION STABILIZES CANCER CELL IDENTITY, REVEALED BY CANCER CELL REPROGRAMMING

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Somatic cell reprogramming is accompanies by dynamic changes of epigenetic modifications and is therefore considered to be a useful tool to induce global epigenetic alteration in cancer genome. However, cancer cells are generally refractory to cellular reprogramming. Here using EWS/ATF1-inducible sarcoma mouse model, we show that EWS/ATF1expression hampers cell fate conversion in EWS/ATF1-addicted sarcoma cells. By withdrawal of the EWS/ATF1 expression, the sarcoma cells can be reprogrammed into induced pluripotent stem cells (iPSCs) capable of teratoma formation and chimeric mouse contribution. Furthermore, blockage of oncogene addiction signals in human cancer cell lines facilitates the early stage of transcription factor (OSKM)-mediated reprogramming. We hypothesized that the disruption of strong oncogenic signal leads to epigenetic alteration which is associated with flexible transcriptional activity, permitting cell fate conversion of cancer cells. Based on this hypothesis, we are currently trying to elucidate the effect of oncogene expression on epigenetic state in cancer cells to understand the mechanism for stable maintenance of cancer cell identity.

F-1161

IMPACT ON BREAST CANCER CELL STEMNESS AND SENESCENCE BY NOVEL ANTICANCER ACTIVITIES IN THE ORMOCARPUM COCHINCHINENSE LEAF EXTRACT

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Breast cancer is one of the most common malignancies in women. Despite significant progress in cancer treatment, disease relapse adversely affects patient survival, because metastatic breast cancers respond poorly to conventional radio- and/or chemotherapy. Emerging evidences indicate that cancer stem cells (CSCs) are the underlying cause of recurrent tumor growth and metastasis that result in treatment failure. Therefore, targeting CSCs could lead to elimination of metastatic and treatment-resistant cancers. As currently available therapeutics are inefficient in eradicating CSCs, there is a requirement to discover novel drugs to treat metastatic breast cancers. Recently, phytocompounds from medicinal plants have received greater attention in the treatment of several cancers. In this context, the leaves of the plant Ormocarpum cochinchinense (OC), which has abundant phytocompounds have been used in traditional Indian medicine to treat various diseases. However, the effects of OC leaf extract (OCLE) on cancer cells are not known. In the present study, we have examined the effects of OCLE on metastatic breast cancer cell line (T47D) proliferation, stemness, spheroid formation and cellular senescence. We assayed cell proliferation by MTT assay, spheroid formation by soft agar assay and senescence by β-galactosidase staining. Furthermore, the mRNA and protein expression of master regulators of stem cell self-renewal and pluripotency (Sox2, Oct-4, Nanog, c-Myc) were studied by real-time RT-PCR and western blot analyses, respectively. Our findings demonstrate that OCLE inhibited proliferation of metastatic breast cancer cells. Most importantly, OCLE reduced the formation and propagation of the spheroids, indicating the loss of stemness and anchorage-independent growth. Consistently, OCLE treatment decreased the expression of pluripotency markers in T47D cells. Interestingly, OCLE treatment also induced cellular senescence. Taken together, our novel findings demonstrate that OCLE has potent anticancer activities as evidenced by reduced growth and stemness, and increased senescence of breast cancer cells, emphasizing the potential use of this plant extract in the treatment of metastatic breast cancer.

F-1163

GATA3 CONTROLS SELF RENEWAL IN THE PROSTATE

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Loss of the tumor suppressor PTEN is a common occurrence in prostate cancer. Here, we show that the transcription factor Gata3 is progressively lost in Pten-deficient mouse prostate tumors. Moreover, 75% of the more aggressive hormone-resistant human prostate tumors show loss of active GATA3. Using a genetic approach, we found that the enforced expression of GATA3 delays tumor progression. This effect is associated with a correction of the aberrant sphere-forming potential of cancerous stem cells to wild-type levels by re-expression of Gata3. Moreover, deletion of GATA3 in normal prostate stem cells enhanced their long term self-renewal capacities in vitro as well as stem cell frequency in vivo. Using RNAseq, we found that loss of GATA3 is associated with a transcriptional change in expression of self-renewal genes. Together, these data establish Gata3 as an important regulator of prostate cancer progression revealing a role for Gata3 in prostate stem cell homeostasis.

F-1165

METALS ACTIVATE OGR1S FROM VARIOUS ANIMAL SPECIES

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G protein-coupled receptors (GPCRs) are activated by various stimuli (lipids, hormones, light, smell, etc.). Human and mouse OGR1s have been recognized as a proton-sensing GPCR. The OGR1s are expressed in various tissues. OGR1deficient mouse showed that OGR1 plays a role in bone metabolisms, endocrine responses, cancer development and progression, and psycho-activity. These functions have been thought to

be mediated by proton through OGR1 activation. It was recently reported that the OGRs were also activated by metals in addition to proton. It opens the possibility that the physiological and pathophysiological roles of OGR1 as described above can also be mediated by the metals. We found zebrafish OGR1 is also a proton-sensing GPCR, however, the kind of metals which activate the GPCR is somewhat deferent from those activate human OGR1. This result prompts us to investigate the metals which activate OGR1s from other species. The coding sequences of OGR1s (human, mouse, rat, pig, chicken, tropical clawed frog and zebrafish) were cloned into an expression vector and expressed in HEK293 cells. The activation of the receptors by metals (manganese, nickel, cobalt and iron) was assessed by SRE-luciferase reporter activities. The result in this study indicates that a caution is necessary when interpreting the result which was obtained from the different species, especially from several animal models for human diseases.

F-1167

HORMONE-DEPENDENT SURVIVAL AND CASTRATION RESISTANCE OF PROSTATE STEM CELLS

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Prostate cancer (PCa) is the most commonly diagnosed cancer in Canadian men. The standard treatment for advanced PCa is androgen deprivation therapy (ADT). As most prostate cells are androgen-dependent, this leads to cell death and regression of the main tumor. Although ADT is initially effective, a recurring and more aggressive form of PCa eventually develops from cancer stem-like cells that are resistant to ADT (ie castration). Similarly, castration in a non-cancer mouse model leads to prostate tissue regression. Moreover, the prostate fully regenerates following administration of testosterone from castration-resistant cells ie prostate stem cells. It is unclear what mechanisms confer castration resistance to normal and cancer stem cells. The IAP (Inhibitor of Apoptosis Proteins) family acts as tight regulators of survival/apoptosis and could be involved in this castration resistance. To test this, we performed castration in both XIAP/BIRC4 and cIAP1/BIRC2 knockout mice. Evaluation of remaining stem cell potential was done by assessing stem cell numbers in regressed prostates by surface staining (FACS) and their in vitro potential using sphere-forming assays. Using tamoxifen inducible CreERT2 lines specific for the luminal (K8CreERT2) and basal lineage (K5CreERT2), we assessed the stem cell activity by lineage-tracing experiments after castration. Our preliminary results indicate that knocking-out XIAP decreases the sphere-forming potential of prostate cells after castration, indicating a potential role in prostate stem cell survival. Comprehending how prostate stem cells can escape the apoptosis pathway will be a crucial step in understanding the acquisition of castration resistance. These results will aid the development of targeted therapies to prevent PCa recurrence.

F-1169

HMGCS1 UP-REGULATES PLURIPOTENCY GENE EXPRESSION AND PROMOTES GASTRIC CANCER PROGRESSION

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Gastric carcinoma is one of the most common cancers and ranks as the third leading cause of global cancer mortality. Gastric cancer patients with distant metastasis have poor prognosis at present and the mechanisms underlying aggressiveness of gastric cancer still remain obscure. A growing body of evidence suggests that metabolic reprogramming, which provides tumor cells with all the metabolites, is one of the hallmarks of cancer cells. The mevalonate pathway involved in cholesterol biosynthesis and protein prenylation is frequently dysregulated in tumors and has been implicated in cancer development and progression. Mounting evidence indicates that the enzyme 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) is one of the controlling points of mevalonate pathway. To screen the genes involved in tumor growth of gastric cancer cells by microarray analysis, HMGCS1 was identified as one of the candidate genes. Our preliminary results showed that levels of HMGCS1 mRNA and protein were enhanced in tumorspheres of SC-M1 and KATO III gastric cancer cells as compared with those in their parental cells. Levels of HMGCS1 mRNA were up-regulated in gastric tumor samples and gastric cancer patients with higher levels of HMGCS1 mRNA had poor survival rate. Levels of mRNAs of pluripotency genes such as Oct4 and SOX-2 were elevated after HMGCS1 overexpression in SC-M1 cells. Activities of reporter genes containing Oct4 and SOX-2 promoters were induced after overexpression of HMGCS1. Additionally, HMGCS1 overexpression promoted migration, invasion, anchorage-independent colony formation, cell viability, tumorsphere formation and xenografted tumor growth of gastric cancer cells, whereas its knockdown had reverse effects. Taken together, these results suggest that HMGCS1 up-regulates pluripotency gene expression and contributes progression of gastric cancer cell.

F-1171

COMPARISON OF EXPRESSION OF MARKERS IN SMALL CELL LUNG CANCER CULTURED IN 2D VERSUS A 3D CANCER ORGANOID SYSTEM

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Small Cell Cancers (SCC) are poorly understood, rare, aggressive cancers, that arise in many organs. These cancers represent highly dedifferentiated tumors that almost always recur from cells that behave like cancer stem cells. Histologically, SCCs are characterized by uniformly small-sized cells with a high nuclear to cytoplasmic ratio. Chemotherapy remains the standard form of therapy and many patients exhibit an initial response to treatment. However, almost all patients relapse and the overall 5-year survival is only around 5%. In this study, we developed a 3D model of SCC using an alginate based inverse opal scaffold and SCC cell lines and patient-derived metastases. We found that the 3D SCC model morphologically recapitulated the tumors seen in patients with sheets of small cells and neural rosette formation. We compared the expression of protein markers EPCAM, NCAM, Synaptophysin, Chromogranin A, vimentin and CD44 by immunofluorescence in the SCC cell lines grown in 2D culture and those grown in the 3D lung organoid system. We found these markers were expressed in the same pattern in the 3D model as in the patient tumor tissue but not in the 2D cultures. This 3D model of SCC holds promise for elucidating the behavior of cancer stem cells in SCC.

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CHROMATIN AND EPIGENETICS

F-1173

DEFINING HUMAN IPSC-DERIVED NEURONAL ACTIVITY-RESPONSIVE GENES AND ENHANCERS

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The majority of human disease-associated variants identified in genome-wide association studies are located in non-coding regions of the genome. These regions are often functionally undefined and many have sequences that are not conserved between human and rodent. Thus, identification and molecular characterization of human cis-regulatory elements driving neural development and plasticity has the promise to profoundly advance our understanding of neurological disease mechanisms. While gene expression and epigenomic analysis of human post-mortem brain samples has allowed for recent advances, these approaches cannot address the dynamic, experience-dependent nature of neuronal gene expression programs. Indeed, programs of neuronal activity-responsive gene expression play key roles in the proper development, refinement, and plasticity of neural circuits, and the dysregulation of these gene regulatory responses have been implicated in a variety of developmental and cognitive disorders. The experimental paradigms that have allowed for interrogation of these regulatory programs in mouse models have been lacking for human, but we attempt overcome this limitation using human fetal brain cultures and human pluripotent stem cell-derived neuronal cultures. We have generated neuronal activity-dependent gene expression profiles in multiple human neuronal culture platforms, and we are employing ChIPseq for both histone modifications and transcription factors to yield a resource map of neuronal activity-responsive regulatory elements across the human genome. Many of these regulatory elements contain SNPs associated with neurological disorders in loci such as CACNB2, KCNB1, ZNFs, and MHC regions. We hope that further functional investigation of these sequences will allow us to understand their possible role in disease and in human evolution.

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F-1175

EFFECT OF EPIGENETIC MODIFICATIONS ON DETERMINATION OF HAIR CELL FATE IN THE COCHLEA

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The sensory hair cells of the inner ear convert mechanical auditory information into a neural signal for transmission to the brain. Whilst it has been established that histone-modifying enzymes dynamically control the chromatin status and modulate the differentiation of multiple lineages, the epigenetic mechanisms modulating hair cell fate specification is poorly understand. We recently developed an in vitro assay to clonally expand and differentiate a subset of cochlear supporting cells expressing Lgr5, into a hair cell-like lineage. This was achieved by treating the cells dissected from postnatal mice with a drug cocktail that manipulates the Notch and Wnt signalling pathways. Here, we modified this protocol by splitting the expanded Lgr5 cells into a 96 well plate (1 cochlea= 12 wells of a 96 well) to screen

the effects of multiple epigenetic drugs on hair cell differentiation. The drug-screening assay revealed that inhibition of the lysine specific-demethylase 1 (LSD1) significantly improved hair cell differentiation in vitro. LSD1 demethylates histone H3 on Lys 4 or Lys 9 (H3K4/K9). Application of the LSD1 inhibitor to cochlear explants obtained from neonatal mice prompted extensive proliferation of supporting cells and hair cell generation in situ. Notably, accumulation of H3K4me2 at the transcriptional start site and enhancer regions of the hair cell specific gene, Atoh1 was detected in the drug treated cells. These findings suggest that modifications of the Atoh1 coding and enhancer regions by H3K4me2 determine the susceptibility of cochlear progenitor cells to adopt a hair cell fate.

F-1177

EPIGENETIC STRATEGIES FOR THE CONVERSION OF HUMAN PLURIPOTENT STEM CELLS INTO HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Inter-conversion of cell states can be achieved by modulating morphogens in a temporal fashion or by over-expression of master transcription factors (TFs), which can directly convert one cell type into another. The promise of converting ES/iPS cells into hematopoietic stem cells (HSCs) remains largely unrealized. We have utilized a combination of morphogen-dependent specification with modulation of key transcription factors in the context of human ES and iPS cells to convert them into HSC or Hematopoietic Stem and Progenitor Cells (HSPC). Over-expression of seven transcription factors (HOXA9, HOXA10, HOXA5, RUNX1, LCOR, SPI1 and ERG) imparted both long-term engraftment (primary and secondary) and multi-lineage (myeloid, erythroid, B- and T-cells) reconstitution in an irradiated immuno-deficient mouse. While genome-wide transcriptomics revealed a close correlation with HSC/HSPC program, lymphocyte specific gene expression signatures seem to be downregulated. This repression seemed to be driven by specific down-regulation of lymphocytic fate determinants- TFs and epigenetic factors (such as EP300, SMARCA2, and others). Current strategies to modulate these factors will be discussed.

F-1179

THE IMPACT OF DNA METHYLATION IN HUMAN NEURAL PROGENITOR CELLS

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DNA methylation is an epigenetic modification that plays a crucial role in gene regulation throughout development and in adulthood. DNA methylation is traditionally thought upon as a repressor of gene expression. However, with the more recent literature it is becoming increasingly clear that the view of DNA methylation and gene regulation is far more complex than previously believed. In mammals, the modification is added and maintained by three catalytically active DNA methyltransferases (DNMTs). DNMT1 is the main enzyme responsible for maintaining DNA methylation during replication, while DNMT3A and DNMT3B are mainly responsible for de novo methylation, but they can also play a role in maintenance. Several knockout studies in mice targeting the DNMTs have shown that DNA methylation is necessary for normal development. In line with this, mutations affecting the DNA methylation in humans are linked to several diseases. In this project we are investigating the role of DNA methylation in human neural progenitor cells (hNPCs). By using CRISPR-Cas9 we have successfully deleted DNA methyltransferase 1 (DNMT1), which is the main enzyme responsible for maintaining DNA methylation during replication. Upon the DNMT1 deletion, the hNPCs lost DNA methylation within a few days, as revealed by immunocytochemistry and bisulphite-PCR. In spite of this, the cells were still viable and continued to proliferate, which is in contrast to proliferative somatic cells in mice. The DNMT1 deleted hNPCs were however unable to survive 30days of differentiation towards neurons. In order to further investigate the cells, they were harvested 10days post the DNMT1-deletion and we are currently investigating changes in both the transcriptome, using RNA-sequencing, as well as the proteome, using mass spectometry. In addition, the changes in DNA methylation will be studied using both bisulphite- and oxidative bisulphite sequencing. Together these approaches will allow us to do correlative analyses between methylation (both 5-methylcytosine and 5-hydroxymethylcytosine) and gene- and protein expression. The results will add to the understanding of the complex nature of DNA methylation in human neural development.

F-1181

EPIGENETIC REGULATION DURING MOUSE INTESTINAL ORGANOID FORMATION FROM STEM CELLS

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A mini-organ called organoid is formed by self-organization of stem cells. The epithelial organoid has been spotlighted as a model for basic and clinical research. The intestinal stem cells are continuously differentiated into a couple of types to replace damaged or lost epithelium cells and also undergo self-renewal for maintenance. The cell fate determination during differentiation of intestinal stem cells should be epigenetically controlled depending on the needs of specific cell type. This epigenetic regulation is a key factor in determining the cell lineage without changing any genetic information. Here we investigated the epigenome and chromatin organization of intestinal organoid derived intestinal stem cells (ISC) as well as embryonic stem cells (ESC). Mouse ESCs were used to differentiate under the several conditions which can specify intestinal lineage and developed into produced small intestinal organoid. By optimizing differentiation condition, intestinal organoid consisting of Lgr5 positive stem cells, Paneth cells and other type of cells was successfully generated and characterized by immunofluorescence imaging and their marker gene expression along with other intestine specific cells. RNA-seq analysis showed that mouse ESCs derived-intestinal organoids exhibit high correlation with adult intestinal stem cell-derived organoids and also much more similar to the intestine. Further analysis of the gene expression profiles and integration of open chromatin structure information could provide some temporal and spatial cues for the differentiation. Our study could provide an applicable potential of massive organoid culture through optimized epigenetic control.

ORGANOIDS

F-1183

BOTTOM-UP ISLET ENGINEERING

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Diabetes Mellitus results from dysfunction of pancreatic islets leading to elevation of blood glucose levels and an increase in morbidity and mortality. In type 1 diabetics, the precipitating event is the loss of insulin producing pancreatic β cells through autoimmune attack. As such, the in vitro production of β cells for use as a cell transplantation therapy has been a major focus of type 1 diabetes research. However, it is unlikely that β cells by themselves will recapitulate the complex biology involved in islet function. Indeed, the three major approaches proposed by the field to regain glycemic control in diabetic patients (bionic pancreas, transplantation of in vitro derived β cells, and production of β cells in vivo through replication or reprogramming) fail to fully account for the complexity of islet endocrine function and focus almost exclusively on the function of the β cell. To this end, we seek to generate human islet organoids from component parts using a bottom-up tissue engineering approach. Here we report the development of separate protocols for generating stem cell-derived α, β and δ cells and subsequently combine these cell types to create islet organoids of defined composition. Stem cell-derived α, β and δ cells exhibit many of the characteristics of their bona fide counterparts including gene expression, hormone secretion, ultrastructure and in vivo function. We also find that combination of stem cell-derived α and β cells in islet organoids exhibit improved function in vitro and in vivo as compared to β cells alone. These studies suggest that stem cell-based products more closely resembling the endogenous architecture and composition of the human islet may be better suited for cell replacement therapy, disease modeling and drug screening efforts.

F-1185

PERSONALIZED RESEARCH: ESTABLISHMENT AND CHARACTERIZATION OF PROSTATE CANCER PATIENT-DERIVED ORGANOIDS AND CELLS

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Several attempts have been made to understand the exact mechanisms underlying the pathogenesis of prostate cancer (PC); however, the currently available cancer models fail to recapitulate the heterogeneity of this tumor, its metastasis, and progression to castration-resistant states. Moreover, many drug candidates that succeed in preclinical models fail to deliver a good outcome in clinical trials, resulting in ineffective patient treatment and misused resources. In this aspect, the development of three-dimensional (3D) organoid culture systems has render it feasible to recap the convolution of organogenesis in vitro, promoting the generation of novel and more representative cancer models. Thus, the aim of this study is to generate patient-specific 3D organoids and cell lines, then characterize these models to identify potential prognostic biomarkers and treatments for PC while correlating the outcome with the collected clinical parameters. In our study, we are employing the R-spondin-1 based organoids technology to generate PC organoids and primary cell lines derived from fresh normal and tumor tissues of patients undergoing radical prostatectomy. Consequently, molecular characterization of the different patient-derived PC organoids and cell lines will be performed, followed by using this model to assess different classical and in-clinical trials drugs. We succeeded in establishing 21 normal and 18 tumour patient-derived organoids, out of a total of 23 patients, and then propagated the established organoids for up to 6 generations. Interestingly, 2D cells were derived from these organoids using the same culture media and were continuously passaged for up to 20 passages so far (more than 3 months). Our newly patient-derived organoids and cells show a typical epithelial phenotype and express CK8 and CK14 (prostate epithelial markers). To the best of our knowledge, this is the first study to address the establishment of primary PC cell lines from organoids, which will likely lead to a comprehensive understanding of mechanisms exploited in PC. Moreover, molecular characterization of this model when combined with pharmacological profiles can aid in predicting a patient's drug response, thus our study represents an attempt to inaugurate what can possibly lead to personalized treatment of PC.

F-1187

COMBINATION OF CRYO-GOLD MEDIUM AND ROCK INHIBITION DRAMATICALLY IMPROVES RECOVERY AND GROWTH OF CRYOPRESERVED MURINE SMALL INTESTINAL ORGANOID

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It has recently been reported that in vitro expanded organoid can be applied to handle gastrointestinal diseases in preclinical models, supporting the establishment of potential organoid-based therapies for restoring damaged intestine. Cryopreservation may be not only a key issue for maintaining organoid culture but also an attractive technique to facilitate the long term storage of large numbers of cells with sustaining the functional properties. In this study, after 3 months of cryopreservation, we found that cryopreserved organoid with CRYO-GOLD freezing medium showed superior recovery compared to that with 10% DMSO. MTT analysis revealed a higher rate of recovery and growth from combination CRYO-GOLD freezing medium and ROCK inhibitor during freezing upon cryopreservation compare with that observed under other conditions reported previously. Moreover, analysis of characteristics of intestinal organoid from freeze-thawed cells showed morphologically well formed-structure representing the crypt and villus, and gene expression of differentiated intestinal epithelial cells during culture. Therefore, these findings suggest that treatment of ROCK inhibitor into CRYO-GOLD freezing medium can significantly improve the recovery of cryopreserved intestinal organoids with the retention of functional properties of cells.

F-1189

A NOVEL METHOD FOR GENERATING SINGLE, UNIFORM, ORGANOIDS FOR HIGH THROUGHPUT SCREENING

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The in vitro organoid model allows for the study of in vivo developmental and biological processes, including tissue renewal, stem cell functionality, and drug responsiveness. As organoids are self-organizing and more accurately resemble the morphological and molecular biology of the tissue they are derived from, there is higher demand for methods to form reproducible organoids for high throughput screening applications.

Currently, many high throughput screens are conducted utilizing 2D monocultures that may not accurately reflect in vivo conditions. Here we demonstrate the formation of gastrointestinal organoids derived from human induced pluripotent stem cells (hiPSC) in the Corning 96 well spheroid microplate. Differentiation into definitive endoderm and intestinal lineage was confirmed by flow cytometry and immunostaining methods.

F-1191

GENERATING MIDBRAIN ORGANOIDS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Midbrain organoids generated from human pluripotent stem cells show promise as models of midbrain developmental diseases. however, have not been established well. In our study, we report here a 3D organoid model of the midbrain containing functional midbrain dopamine neurons. We developed a protocol to generate iPS cells into a midbrain organoid that contains distinct layers of dopamine neuronal cells. Moreover, we are able to demonstrate the characteristics of mature midbrain dopamine neurons in the 3D midbrain-organoids. Remarkably, our midbrain organoids produced dopamine granules that were structurally similar to those isolated from midbrain substantia nigra tissues. Thus our midbrain organoid bearing features of the midbrain may provide a practical in vitro system to study the midbrain and Parkinson's diseases.

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F-1193

CHARACTERIZATION OF DOPAMINERGIC NEURONS IN ORGANOID DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) have created an unlimited source of material for tissue engineering, cell therapy and complex diseases modeling in vitro. However, one of the drawbacks is also the generation of a heterogeneous population after the differentiation process. In this study, we established a midbrain organoid culture model derived from induced pluripotent stem cells that contain a higher and purer population of dopaminergic (DA) neurons. This was achieved with the selection of floorplate progenitor cells by sorting for CORIN-positive cells in the DA neurons induction stage before Matrigel encapsulation. Cells in the organoid were displaying a midbrain-like phenotype characterized by the expression of tyrosine hydroxylase (TH) and a neuronal marker, MAP2. Dopaminergic neurons derived from Parkinson's disease (PD) iPSCs in organoid culture model also contain alpha-synuclein aggregations which recapitulate the disease phenotype of PD. In contrast, alpha-synuclein aggregations were not obvious in our two-dimensional (2D) culture model.

F-1195

DE NOVO HAIR FOLLICLE INDUCTION FROM MOUSE PLURIPOTENT STEM CELL-DERIVED SKIN ORGANOIDS

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In mammals, hair follicles are produced by synchronized interactions between the surface epithelium and dermal mesenchyme during embryonic developmental stages. It remains unclear how to recapitulate hair induction in pluripotent stem cell-derived skin equivalents, which could provide a power platform for basic research or drug discovery. Hair follicle induction in vitro has been demonstrated using primary cells extracted from embryonic skin, but these approaches are poorly defined and require serum. Here, we describe the generation of skin organoids from a homogeneous population of mouse pluripotent stem cells in 3D culture by co-inducing surface epithelium and mesenchymal cells under serum-free conditions. The generated skin organoids are composed of Keratin (Krt)5+ Krt10+ Krt15+ Filaggrin+ multi-layered epidermis and a CD34+ PDGFRα+ fibroblast-like cell containing dermis, reminiscent of mouse embryonic skin. Moreover, the skin organoids were folliculogenic, generating follicles with Sox2+ dermal papilla cells and Krt15+ hair bulge stem cells. Additional pilosebaceous unit features, such as lipid-rich sebaceous gland and adipocytes and Itga8+ αSMA+ arrector pili-like cells, were also present in the skin organoids. Finally, we confirmed that skin organoids could be produced from multiple pluripotent stem cell lines. Thus, our mouse pluripotent stem cell-based culture system of integumentary development offers a versatile model for in-depth study of the mechanisms of hair follicle induction, evaluating hair growth drugs, or modeling skin diseases.

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TISSUE ENGINEERING

F-1197

ADVANCED APPROACHES TO EMPLOYING CHEMICAL AND PHYSICAL CUES FOR MESENCHYMAL STEM CELL DIFFERENTIATION

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Stem cell differentiation often results in heterogeneous cell populations, reducing stem cell therapeutic efficacy. Chemical and physical cues are both known to modulate differentiation. Here, we present two unique approaches we investigated to employ either chemical or physical cues to direct stem cell differentiation. First, we will describe the temporal effects of geometric confinement on stem cell differentiation, which is currently not understood. We hypothesized that the time spent in confinement influences lineage specificity. Thus, we have developed intrinsically degradable protein patterns using alkanethiol self-assembled monolayers (SAMs) on gold (Au). By changing Au thickness (4, 8, or 10 nm), we can control SAM degradation rates. X-ray photoelectron spectroscopy of hydrophilic SAMs showed a significant loss of the thiol peak over time at 5 days for 4 nm Au (~23%) and 10 nm Au (~22%), and at 7 days for 8 nm Au (~7%). Mass spectrometry, atomic force microscopy, and water contact angle measurements revealed a similar pattern. Human mesenchymal stem cells (HMSCs) seeded on fibronectin-coated glass surrounded by bioinert SAM regions depicted migration behavior that directly correlated with these results – 5 days for 4 and 10 nm Au and 7 days for 8 nm Au (53 ± 11.7%, 2.5 ± 0.04%, and 16.0 ± 12.5% migration from the fibronectin to the bioinert regions, respectively). Multi-cell containing patterns on 8 nm Au (which degraded at 7 days) demonstrated that patterned cells resulted in a greater adipogenic response from day 7 to day 14 ($p=0.03$) than non-patterned controls ($p=0.40$). The effect of single-cell patterns on our degradable surfaces is currently being investigated. With respect to chemical cues, we have focused on kartogenin (KGN). KGN is a newly discovered synthetic small molecule known to induce HMSC chondrogenesis by binding to filamin A, causing the nuclear localization of core binding factor β (CBFβ). KGN's hydrophobicity, however, limits its application in regenerative medicine. We have increased KGN's solubility by conjugating it to the naturally occurring polymer, hyaluronic acid (HA). We predict that known interactions between HA and CD44, which can recruit filamin A, will improve KGN's efficacy by localizing KGN to its known site of action in the cell and are currently investigating this hypothesis.

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F-1199

NEURAL STEM CELL-LADEN MULTICHANNEL BRIDGES SUPPORT AXON REGENERATION AND ENDOGENOUS NEUROGENESIS FOLLOWING SPINAL CORD INJURY

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Loss of nerve function due to axon damage and demyelination following spinal cord injury (SCI) presents insurmountable challenges to axon regeneration through the injury without intervention. Multichannel poly(lactide-co-glycolide) (PLG) bridges offer a promising biomaterial platform to foster growth of nerve bundles through the injury. The high degree of porosity within these bridges supports cell infiltration and integration of the bridge into surrounding tissue, while channels guide axons from the rostral tissue across the injury, with re-entry into the caudal tissue. The pores and channels facilitate delivery of therapeutic factors or cells that can enhance the regenerative potential. Neural stem cells (NSCs) promote nerve regeneration through a variety of mechanisms, including neuronal cell repopulation and neurotrophic factor release. We investigated the ability of the architectural support of PLG bridges to provide a protective reservoir that supports the survival and function of NSCs, leading to a synergistic increase in axon regeneration and re-myelination. Mouse embryonic day 14 (E14) and adult spinal cord NSCs expressing green fluorescent protein (GFP) were cultured on PLG bridges then transplanted into a syngeneic lateral T9-10 hemisection. GFP+ cells were evident in the bridge and contralateral tissue 8 wks post-injury with improved survival of E14 NSCs compared to adult NSCs within the bridge. Approximately 30-40% of surviving GFP+ cells differentiated into neurons or oligodendrocytes. Endogenous neurogenesis and oligodendrogensis were evident in NSC-loaded bridges, with an ~20-fold and ~7-fold increase in neurons and oligodendrocytes, respectively, in NSC-loaded bridges compared to blank bridges. Only E14 NSC-loaded bridges enhanced nerve regeneration compared to blank bridges, resulting in a 3.3-fold increase in axon elongation (1674 v 497 axons/mm2) and 3.6-fold increase in myelination (~30% of axons). Modest improvements in locomotion were indicated for both NSC-loaded bridge groups including an earlier ability to perform ipsilateral hindlimb stepping. Together these results suggest that combining bridges

that mimic spinal cord architecture with regenerative NSCs can lead to synergistic regenerative gains due in part to neurogensis, axon growth, and re-myelination.

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F-1201

ECM PROTEIN LAMININ ENHANCES HUMAN MESENCHYMAL STEM CELL PARACRINE FUNCTION VIA AVB3/CD61 INTEGRIN TO REDUCE CARDIOMYOCYTE APOPTOSIS

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Myocardial ischemia (MI) results in extensive cardiomyocyte death and reactive oxygen species (ROS)-induced damage in an organ with little or no regenerative capacity. Although the use of adult bone marrow mesenchymal stem cells (BMMSCs) has been proposed as a treatment option, the high cell numbers required for clinical use are difficult to achieve with this source of MSCs, and animal studies have produced inconsistent data. We recently demonstrated in small and large animal models of acute MI that the application of human term placenta-derived multipotent cells (PDMCs), a fetal-stage MSC, resulted in reversal of cardiac injury with therapeutic efficacy. However, the mechanisms involved are unclear, making it difficult to strategize for therapeutic improvements. We found that PDMCs significantly reduced cardiomyocyte apoptosis and ROS production through the paracrine factors GRO-α, HGF, and IL-8. Moreover, culturing PDMCs on plates coated with laminin, an extracellular matrix (ECM) protein, resulted in significantly enhanced secretion of all three paracrine factors, which further reduced cardiomyocyte apoptosis. The enhancement of PDMC paracrine function by laminin was mediated through αvβ3 integrin, with involvement of the signaling pathways of JNK, for GRO-α and IL-8 secretion, and PI3K/AKT, for HGF secretion. Our results demonstrated the utility of PDMC therapy to reduce cardiomyocyte apoptosis through modulation of ECM proteins in in vitro culture systems as a strategy to enhance the therapeutic functions of stem cells.

F-1203

PEDF SHORT PEPTIDE CAN EXPAND RAT TENDON STEM CELL IN CULTURE AND FACILITATE THE REGENERATION OF INJURED RAT TENDON

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Tendon injury occurred frequently during sport activity. Tendon injury is usually not healed by forming tendon fiber but rather by forming scar tissue. This will affect tendon weight bearing capability and disable the patient. Pigment epithelium derived factor (PEDF) has been shown to be functional in reparation of several damage tissue such as liver and cornea. In this study a short peptide derived from N-terminal of PEDF is employed to test the efficacy for facilitating tendon regeneration. Ankle tendon of rat was injured by needle penetration and peptide delivery is achieved by local injection of a mixture of peptide and alginate gel in order to achieve sustained release. Histology section of tendon sample harvested two months later revealed that parallel tendon fiber with moderate collagen accumulation is seen in peptide treatment group, indicating quality tendon healing. However, tendon from vehicle treatment group showed disorganized tendon fiber with heavily laden collagen accumulation, indicating scar formation. PEDF peptide healed tendon can sustain higher weight bearing. In cell culture, tenocyte can be induced into proliferation by PEDF peptide. Moreover, PEDF peptide in primary culture of tendon tissue can maintain the population of tendon stem cell which expressed the stem cell markers Oct-4, SSEA-4, and nucleostemin. These evidence indicated that by maintaining tendon stem cell and tendon cell proliferation, PEDF peptide can facilitate the healing of injured tendon.

F-1205

A GELATIN-SULFONATED SILK COMPOSITE SCAFFOLD ENHANCES SKIN REGENERATION

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One of the key problems hindering skin repair is deficiency of dermal vascularization and difficulty of epidermis regeneration, making it challenging to fabricate scaffolds that can biologically fulfill the requirements for regeneration of skin injuries. To solve this problem, we developed a gelatin-sulfonated silk composite scaffold based on three dimensional printing and incorporated the scaffold with basic fibroblast growth factor (FGF-2) through a sulfonic acid group (SO3)(3DG-SF-SO3-FGF). The efficacy and mechanism

by which the 3DG-SF-SO3-FGF scaffolds promote skin regeneration were investigated both in cell culture and a full-thickness skin defect model. The histological results showed that gelatin-sulfonated silk composite scaffolds promoted granulation, and incorporation of FGF-2 significantly enhanced the regeneration of skin-like tissues after implantation in rat skin defects for 14 and 28 days. Further investigations demonstrated that 3DG-SF-SO3-FGF scaffolds might stimulate the vascularization of dermis. These findings suggest that incorporation of FGF-2 into the 3D printed scaffolds is a viable strategy for skin regeneration.

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F-1207

SHEAR STRESS ENHANCES ARTERIAL ENDOTHELIAL DIFFERENTIATION OF STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH VIA VEGF-NOTCH-DLL4 SIGNALING

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This study aim to evaluated the impact of shear stress on the expression of venous and arterial markers in ECs that were derived from human exfoliated deciduous teeth (SHED), and uncovered the molecular mechanism. SHEDs were evaluated before induction, the expression of cells surface markers, CD90, CD73, CD45, STRO-1, and CD105 were assessed by flow cytometry. Additionally, osteo/odontogenic, adipogenic, and neurogenic differentiation assays were conducted in the respective induction media. For induction, SHED were treated with fluid shear stress for 2 h alone or combined with VEGF stimulation for 24 h, Real-time PCR and Western blot assay were performed to investigate RNA and protein expression. Tubule formation assay were performed to analyze endothelial differentiation of SHED after fluid shear stress induction. Fluid shear stress of different values (4 Dynes/cm2 and 16 Dynes/cm2) significantly increased the mRNA expression of EphrinB2, VEGF, Dll4, and VEGFR2 in SHED after induction for 2 h. Protein expression levels of ephrinB2 were significantly up-regulated while EphB4 expressions were significantly down-regulated compared to the untreated groups after induction. CD31 and VEGFR2 protein expression levels were significantly up-regulated 12 h and 24 h after induction compared to the untreated groups. Tubule formation assay demonstrated that fluid shear stress promoted the formation of vascular-like structures compared to untreated groups. Fluid shear stress can induce arterial endothelial differentiation of SHED in vitro, possibly through VEGF-Notch-Dll4-EphrinB2 signaling.

F-1209

EFFECTS OF CONCENTRATE GROWTH FACTORS ON THE OSTEOGENIC POTENTIAL OF PERIODONTAL LIGAMENT STEM CELLS

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Some kinds of growth factors play an important role in stem cell osteogenic induction. Concentrated growth factors (CGFs) is the third generation of platelet concentrate products .It uses variable rpm from 2400-2700 rpm to separate cells in the venous blood, resulting in fibrin rich blocks containing much larger, richer growth factors than PRF. However , few studies was done to investigate the effects of CGF in stem cell osteogenic induction. In order to investigate the effects of CGFs on the osteogenic potential of periodontal ligament stem cells (PDLSCs), we did this study. PDLSCs were isolated from the intermediate 1/3 of the root of premolar teeth of volunteers. CGFs were prepared from venous blood drawn from volunteees. PDLSCs were culcured and divided into two groups: control group and experimental group (induced by CGF). The PDLSCs and CGFs used in experimental group came from the same donor. The mineralized nodules were counted , alkaline phosphatase activity detection and qPCR were conducted . Cell count, ALP activity, mineralized nodule numbers were invested in both control and experimental group. The experiments aforementioned showed that the CGFs might have positive effect on the osteogenic potential of PDLSCs.

F-1211

EX VIVO DIFFERENTIATION OF UMBILICAL CORD STEM CELLS INTO CELLS EXPRESSING NUCLEUS PULPOSUS MARKERS

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Intervertebral disc (IVD) degeneration due to age, trauma, or genetic disease is one of the major causes of severe low back pain. Current treatments address symptomatic pain rather than the cause of the disease. As such, there is a need for regenerative therapies that restore native tissue structure, cellularity and mechanical function. IVD degeneration is characterized by changes

in the cell populations, and the subsequent loss of extracellular matrix (ECM) of the nucleus pulposus (NP); whereby, notochordal cells, which produce NP, are replaced by chondrocyte-like cells resulting in fibrous tissue, and ultimately a loss of proteoglycans and osmotic properties of the disc. In this study, we investigated the differentiation of hydrogel encapsulated human umbilical cord stem cells (CSCs) into nucleus pulpous producing cells (NPCs) using an ex vivo rabbit disc model of degenerative disc disease (DDD). Results indicated that encapsulated cells in 3-D hydrogel scaffolds remained viable in ex vivo IVD culture and appeared fibroblastoid in contrast to a round morphology in vitro, signifying integration into the tissue. Moreover, injection of encapsulated CSCs and NPCs into the ex vivo disc environment stimulated differentiation into cell types capable of improved ECM production including sulfated glycoaminoglycans (sGAG) when compared to injection of cells alone. Encapsulated CSCs and NPCs also showed higher expression of chondrogenic markers, SOX9, COL2, and AGGRECAN, as well as putative NP markers, FOXF1, KERATIN19, and VIMENTIN as determined by quantitative real-time PCR and immunostaining. Overall, this study demonstrates the potential of CSCs and their derivatives for regeneration of the NP using tissue engineering and cell therapy techniques.

F-1213

SYNERGISTIC ENHANCEMENT OF ENDOTHELIAL DIFFERENTIATION WITH MICROTOPOGRAPHY AND VEGF ON HUMAN MESCENCHYMAL STEM CELLS FROM DONORS OF DIFFERENT GENDERS AND AGES

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Cell therapy for vascular damage has been showing promises as alternative therapy for endothelial dysfunctions since the discovery of the endothelial progenitor cells (EPCs). However, isolated EPCs from peripheral blood yield low cell amounts and alternative cell source must be explored. The aim of this study was to investigate the influence of topography on the endothelial differentiation of an alternative cell source – human bone marrow mesenchymal stem cells (hMSCs). In a systematic screening of variety of patterned surfaces using an array of topography on a MultiARChitecture (MARC) Chip and different medium compositions, convex microlens pattern in combination with the vascular endothelial growth factor (VEGF) enriched medium was shown to be the most efficient on the endothelial differentiation. The synergistic enhancement yielding up to 10% of CD34+CD133+KDR+ marker expressing differentiated hMSCs as analyzed by flow cytometry, compared to 1.1% from unpatterned control enriched with VEGF, or 1.1% from microlens pattern in growth medium. The quantified tube-like structures in the Matrigel assay in vitro indicated a vasculogenic potential of these endothelial progenitor-like differentiated hMSCs that was investigated further in a Matrigel plug assay in vivo in a rat for seven days. The hMSCs cultured on the convex microlens in medium enriched with VEGF, implanted in a Matrigel plug in a rat, showed the highest capillary density, the highest human endothelial cell marker Ulex Europaeus agglutinin-positive (UEA-1+) capillary density, as well as the highest UEA-1+ cell survival density that were not included in the vasculogenesis. These findings indicate the active participation of the vasculogenic hMSCs in the vasculogenesis. The endothelial differentiation of hMSCs using this synergistic combination of microlens and VEGF enriched medium was also demonstrated in hMSCs from different male and female donors. Moreover, when comparing the differentiation of hMSCs from young donors and old donors (>55 years old), the synergistic effect of differentiation was more significant in hMSCs from younger donors. The culture platform with combination of topography and biochemical cues could generate vasculogenic cell populations that may prove useful in vascular damage or other clinical applications.

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ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

F-1219

A RE-INTERPRETATION OF THE CANADIAN PROHIBITION ON HUMAN GERM LINE EDITING

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The rapid evolution of gene editing technologies and the discussions occurring both nationally and internationally to determine how best to regulate and address its promises and challenges, have made clear that Canada should be part of the debate. Through an expert consultation of the author group, both the status of human gene editing in Canada and potential avenues for policy recommendations and modifications to the Canadian regulatory framework were discussed. Since 2004, there has been an accepted legal interpretation of section 5(1)(f) of the Assisted Human Reproduction Act (AHRA). This was questioned by the group, along with the scope of the criminal sanctions tied to altering the human germ line. Hence the need to assess: 1) whether the AHRA has been correctly interpreted since coming into force in 2004; 2) whether research applying gene editing technologies to the human germ line should be subject to criminal prohibitions; and 3) if not, what type of regulation or approach would be most appropriate and perhaps serve as a model for other countries? The group identified the need to: revisit current laws and policies; provide a principled approach to policymaking; inform and engage the public; and determine the type of necessary oversight.

F-1221

THE UNIQUE ETHICAL ISSUES IN INDUCED PLURIPOTENT STEM CELL RESEARCH

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It is a common refrain that "iPSC technology avoids the ethical issues encountered with hESCs" because hESC technology requires the destruction of human embryos. This poster closely examines that supposition and presents ethical issues unique to iPSCs that warrant serious consideration. Ethical/legal issues concerning the rights of fertility patients to decide the disposition of their embryos, as well as considerations of re-identification of pluripotent stem cell donors will be discussed. The ramifications of cell line immortality and intellectual property will also be presented.

Funding Source: Nonfederal funds from the University of Washington Institute for Stem Cell and Regenerative Medicine and The Ellison Foundation

F-1223

CLINHIPS: A SCIENTIFIC, ETHICAL AND COMPARATIVE LEGAL ANALYSIS OF THE CLINICAL APPLICATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN GERMANY AND AUSTRIA

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The project ClinhiPS analyses the clinical application of human induced pluripotent stem cells (hiPSCs) from a scientific, ethical and comparative legal perspective. hiPSCs were first generated in 2007 from adult human cells through transduction of four transcription factors, namely Oct4, Sox2, c-Myc and Klf4. hiPSCs are fully differentiated body cells (such as dermal fibroblasts) that have been reprogrammed in the laboratory to take on the pluripotent properties of human embryonic stem cells, i.e., stem cells that have the potential to develop into any of the 200 different human cell types; resulting in great excitement across research laboratories globally. hiPSCs are already utilised as a powerful tool for disease modelling and drug testing, and they are clearly paving the way towards clinical application.

In order to successfully translate research results into clinical practice, it is essential to identify and examine the scientific, ethical and legal issues facing the clinical application of hiPSCs at an early stage. Consequently, this project analyses the scientific and practical background for clinical hiPSC application, thereby examining the overall process from hiPSC generation to the cell therapy itself. It also examines the ethical challenges associated with clinical implementation of hiPSC therapies. The project aims to provide an analysis of relevant stakeholders and ethical tools necessary for regulatory oversight to safeguard patients' and public interest in medically innovative patient care, with the results feeding into the development of ethical guidelines. In addition, the project compares German and Austrian law, thereby showing the legal similarities and differences between offering a future hiPSC therapy in a German and an Austrian clinic. It will reveal legal defects and gaps in the current regulatory approach to the clinical application of hiPSCs in Germany and will provide recommended actions to the German legislator. The project aims to establish a comprehensive guide to the clinical application of hiPSCs for all stakeholders involved (e.g., doctors, researchers and patients).

Funding Source: Sponsored by the Federal Ministry of Research and Education

CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

F-1225

SAFETY AND EFFICACY OF HUMAN EMBRYONIC STEM CELL-DERIVED OLIGODENDROCYTE PROGENITOR CELLS (AST-OPC1) IN PATIENTS WITH SUBACUTE CERVICAL SPINAL CORD INJURY

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AST-OPC1 is a cryopreserved population of cells derived from human embryonic stem cells which contains oligodendrocyte progenitor cells and other characterized cell types. The initial safety of AST-OPC1 was evaluated in a phase 1 clinical trial that enrolled 5 patients with neurologically complete T3-T11 thoracic spinal cord injury (SCI). Based on favorable 5 year safety data, an open-label phase 1/2a clinical trial (SCiStar) is underway to evaluate the safety and activity of escalating doses of AST-OPC1 in patients with severe (AIS-A or AIS-B) C5-C7 SCI. Doses of 2x106, 1x107 and 2x107 AST-OPC1 are being tested in 5 cohorts of patients. Subjects are followed by the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) neurological exam and other assessments including MRI to determine safety and activity. Thirteen subjects have been dosed to date. Cohorts 1 (AIS-A, 2x106 cells) and 2 (AIS-A, 1x107 cells) are complete with 3 and 6 subjects, respectively. Enrollment in cohorts 3 (AIS-A, 2x107 cells), 4 (AIS-B, 1x107 cells) and 5 (AIS-B, 2x107 cells) is progressing. The results to date from patients in cohorts 1 and 2 indicate no safety issues associated with the delivery of AST-OPC1 or the cells themselves. All patients in cohorts 1 and 2 have experienced improved upper extremity motor function as assessed by upper extremity motor scores (UEMS) and neurological level of motor function. The average UEMS improvement for the 3 patients in cohort 1 was 6.3 (range 3-10) points at 6 months, with each patient demonstrating one neurological level improvement in motor function. The 5 patients in cohort 2 who have completed 6 months of follow-up, recovered an average 9.8 (range 6-16) motor points with continued improvement for the three subjects who have reached 9 months of follow-up. Two of 5 patients followed in cohort 2 for at least 180 days achieved a 2 motor level neurological improvement. The motor function improvement in patients in cohort 2 compares favorably with a closely matched historical group of 62 traumatic SCI patients derived from the EMSCI (European Multicenter Study about Spinal Cord Injury) database of over 3300 patients. The data to date demonstrate the safety of AST-OPC1 and provide encouraging early evidence of improvements in arm, hand and finger function.

Funding Source: Asterias Biotherapeutics, California Institute of Regenerative Medicine (CIRM)

F-1227

PRODUCTION OF CLINICAL GRADE TEMPORARY EPIDERMAL SUBSTITUTE OBTAINED FROM HESC DERIVED KERATINOCYTES FOR THE TREATMENT OF SICKLE CELL LEG ULCERS: A CHALLENGE FOR REGENERATIVE MEDICINE

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Skin is the largest organ of the body involved in self-protection against external damages. Epidermis,

the upper layer of the skin is mainly composed of keratinocytes organized to form a physical barrier at the interface of the environment. Some of diseases associated to genetic mutations or not could weaken this protection and lead to the disruption of skin integrity. Cell therapy approaches using adult keratinocytes are currently envisaged however these cells present limited proliferative capacities and variability in genetic background. Access to an unlimited source of embryonic pluripotent stem cells (hESC) will aim at overcoming these limitations since these cells are available in unlimited quantities thanks to their unlimited proliferation capacity and their pluripotency. In this context, a protocol allowing the generation of keratinocytes from hESC able to perform functional pluristratified epidermis was developed. In the perspective of a human clinical application, the entire protocol have been optimized and adapted following good manufacturing practice (GMP) conditions from a clinical grade hES cell line (RC9) obtained at the Biotech Company Roslin Cells. A quality control of the keratinocytes was established. These controls include the checking for contaminations, karyology, and viability. Specific controls such as the analyses of the expression of keratinocytes markers and the absence of pluripotency markers were performed to verify the quality of the keratinocytes cells bank. In addition, a clinical grade support was selected for this capacity to allow the formation of a pluristratified epidermis in vivo. To certify the safety in human therapy using cells derived from hESC, pre-clinical experiments will be performed to analyze the theoretical risk of a cell shedding to distant organs, and tumorigenicity due to residual pluripotent cells.

F-1229

CHARACTERIZATION OF CRYOPRESERVED HUMAN DENTAL PULP STEM CELLS PREPARED UNDER XENOGENEIC FREE-CULTURE CONDITIONS

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Dental pulp-derived mesenchymal stem cells (DPSCs) have shown great potential for cell-based therapies; however, the use of fetal bovine serum (FBS) and dimethyl sulfoxide (DMSO) are obstacles for clinical application because of immunoreaction or cytotoxicity. In this study, we aimed to characterize cryopreserved DPSCs using an FBS-free culture medium and DMSO-free cryopreservation medium. DPSC isolation at passage (P) 0 and subsequent cultivation were performed using PRIME-XV○R MSC Expansion XSFM (XFM). Following subculture at P1, DPSCs were cryopreserved using CryoScarless○R DMSO-Free, and designated c-DPSCs; non-cryopreserved cells were designated n-PDSCs. After 1–3 month cryopreservation, the cells were recovered and cultured until P3 or P4 for the following stem cell characterization experiments: proliferative assay, population doubling time calculation, cell cycle analysis, flow cytometry, RT-PCR, and multi-differentiation induction experiments. Both groups of cells exhibited a typical spindle-shaped fibroblastic morphology. The proliferation levels and the ratio of fractionated cells that underwent cell division (the S and G2 phases) showed similar profiles in both cell groups during the culture period. All the cells were positive for CD44, CD90, CD105, and STRO-1 via flow cytometry; and expressed the genes encoding osteogenic (Vimentin, Runx2, and Type I collagen), neurogenic (Nestin), and stem cell markers (Nanog, Oct3/4, and Sox2), as determined by RT-PCR. c-DPSCs showed multi-differentiation capabilities, equivalent to that of n-DPSCs. c-DPSCs exhibited no cytotoxic effects and retained stem cell properties regardless of DMSO-free cryopreservation. FBS-free and DMSO-free culture protocols for DPSC handling therefore hold a promise of safe and reliable cell processing for future cell-based therapies.

F-1231

ENSURING POST-GRAFTING SAFETY RIDDANCE OF STEM CELL THERAPY PRODUCTS WITH THE TK-SUICIDE GENE STRATEGY: CRISPR-CAS9 MEDIATED KNOCK-IN IN AAVS1 OF TK TRANSGENE

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An important safety concern for cell therapy with human pluripotent stem cells (hPSC) remains the potentially uncontrolled proliferation of grafted cells. This phenomenon can lead, amongst other issues, to teratoma/tumor formation or overgrowth. This is particularly relevant for hPSC-grafts in Huntington's disease (HD). Indeed, a current clinical-grade medicinal product transplanted in HD patients (fetal ganglionic eminence-derived graft) is seen to proliferate after transplantation. To deal with possible adverse effects, we tested a safety system based upon genetic engineering of the grafted cells with the Herpes simplex type 1 thymidine kinase

(TK) suicide gene. We generated several transgenic human and Monkey PSC lines expressing HSV1-TK using lentiviral, plasmidic vectors (random integration) or CRIPSR-Cas9-mediated knock-in (AAVS1-locus specific integration). Efficacy of such TK-based safety system was first studied in vitro using undifferentiated PSCs, post-mitotic neuronal derivatives and/or proliferative immature neural derivatives to challenge the system. In vitro dose response analyses of GCV sensitivity of TK+ hPSC lines revealed that proliferative hPSC and hPSC-derived neural cells are highly sensitive to GCV (IC50 in sub-micromolar range). In contrast, post-mitotic neurons generated from TK+ lines are insensitive to GCV. We then explored the therapeutic potential of such system in an allo-transplantation context in non-human primate. We showed that the proliferation of TK+ proliferative neural grafts, in the striatum of Macaca fascicularis, can be stopped by a delayed treatment with val-GCV. MRI longitudinal follow-up and histological characterization of graft composition and differentiation showed in addition that neuronal content was not affected by val-GCV treatment. Next, we shall explore a GMP-compliant transgenic approach using the CRISPR-Cas9 technology as a gene editing tool coupled with MACS as a GMP-compliant option to select transgenic hPSC lines. Overall our results suggest that safety issues linked to graft overproliferation in the brain can be managed using a TK-suicide gene system and that GMP-compliant technologies may be available to produce TK+ lines without compromising their differentiation or biological activity in vivo.

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GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

F-2001

GENOME TRANSFER PREVENTS FRAGMENTATION AND RESTORES DEVELOPMENTAL POTENTIAL OF DEVELOPMENTALLY COMPROMISED POSTOVULATORY AGED MOUSE OOCYTES

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To investigate the roles of nucleus or cytoplasm elements in oocyte fragmentation and poor developmental potential, we used post-ovulatory aged oocytes as a model and performed nuclear transfer both at MII and pronucleus stages. Post-ovulatory aged oocytes over 20h post oocyte retrieval showed defect at MII alignment and chromosomal segregation error at anaphase. The transfer of nuclear genomes from post-ovulatory aged to fresh MII oocytes showed less defect at MII alignment at anaphase, but fresh to post-ovulatory aged oocytes showed severe defect both at metaphase and anaphase. 60.0±7.4% post-ovulatory aged to fresh activated oocytes developed to blastocyst stage and embryonic stem cells were isolated, but none of fresh to post-ovulatory aged activated oocytes developed (fresh embryos; 72.1±19.6%, post-ovulatory aged oocytes; 0.0±0.0%, respectively). IVF using post-ovulatory aged to fresh oocytes resulted in full-term development and viable pups have been obtained. In addition, to investigate the roles of transcriptional factors in oocyte aged in vitro process and their subsequent development capability following activation, we reconstructed activated oocytes with pronuclei from post-ovulatory aged and fresh oocytes by pronuclear transfer. The pronucleus transfer between activated oocytes showed that 50.6±6.6% post-ovulatory aged to fresh activated pronucleus oocytes developed to blastocyst stage, but 3.0±0.3% of fresh to post-ovulatory aged activated pronucleus oocytes developed. The increased developmental potential is explained primarily by correction of abnormal cytokinesis at anaphase of meiosis and mitosis, by a reduction in chromosome segregation errors, and by normalization of the localization of chromosome passenger complex components survivin, and of cyclin B1. These observation demonstrated that cytoplasm is more crucial than nucleus to aging process. Nuclear transfer can prevent abnormal cytokinesis and fragmentation of fertilized eggs at mitosis, and restore developmental potential to mouse eggs aged in vitro.

F-2003

THE ROLE OF JMJD3 IN THE REGULATION OF SPERMATOGONIAL STEM CELLS

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Spermatogenesis is continuous process, because spermatogonial stem cells (SSCs) can maintain themselves as well as provide differentiated progenies. The maintenance of SSC compartment is supported by not only self-renewal of stem cells but also fragmentation of differentiating spermatogonia through abscission of intercellular bridges in a random and stochastic manner. The molecular mechanisms that regulate this reversible developmental lineage still remain unclear. We have shown that histone H3 lysine 27 (H3K27) demethylase, JMJD3 (KDM6B), regulated the fragmentation of spermatogonial cysts. Although lack of JMJD3 in germ cells did not affect differentiation of spermatogonia, JMJD3 null mice have larger testes and sire offspring for a longer period compared to controls, likely secondary

to increased and prolonged maintenance of the spermatogonial compartment. The absence of JMJD3 induces frequent fragmentation of spermatogonial cysts by abscission of intercellular bridges. Here we show and discuss that molecular mechanisms of JMJD3 to regulate fragmentation of spermatogonial cyst by analyses of downstream target of JMJD3. These results suggest that JMJD3 controls the spermatogonial compartment through the regulation of fragmentation of spermatogonial cysts and this mechanism may be involved in maintenance of diverse stem cell niches.

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PLURIPOTENCY

F-2005

TRANSCRIPTIONAL NETWORKS VARIATIONS DURING CELL CYCLE PROGRESSION IN HUMAN EMBRYONIC STEM CELLS

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Differentiation and cell cycle regulation in stem cell have a key function for embryonic development, organ homeostasis and tissue repair. Recent results have shown that these two mechanisms are intrinsically connected. Indeed, cell cycle machinery directly controls maintenance of pluripotency and initiation of differentiation. More precisely, the cell cycle regulator Cyclin D appear to control the transcriptional activity of Activin/Nodal signalling during progression of cell cycle in human Embryonic Stem Cells (hESCs). As a consequence, hESCs can only differentiate into endoderm in the Early G1 phase when Cyclin Ds are expressed at low level. These results show the mechanisms by which the cell cycle defines differentiation propensity of stem cells. However, these observations also imply the existence of interplays coordinating extra cellular signalling pathways with epigenetic, chromatin structure and transcriptional networks during cell cycle progression and these mechanisms remain to be fully uncovered. Here, we utilised the FUCCI reporter system combined with ATAC-Seq to analyse chromatin dynamics during cell cycle progression in hESCs. Furthermore, we performed ChIP-Seq to define the genomic location of transcriptional regulators during cell cycle progression as well as RNA-Seq to confirm the gene expression pattern. The integration of these data show that in hESCs the chromatin status is highly dynamic as core pluripotency transcription factors and epigenetic modifiers genomic location. We also showed that hESCs in the Late G1 phase accumulate transcripts that are important for differentiation and development; therefore indicating this phase represents a unique portion of the cell cycle for cell fate decisions. Taken together, these results demonstrate the existence of diverse transcriptional networks in the different phases of the cell cycle. We hypothesise that these dynamic modifications are necessary to prime hESCs for different cell fate choices allowing a diversity of differentiation impossible otherwise. Overall these mechanisms could be easily transferable to somatic stem cells and thus have a key function not only in vitro but also in adult tissue and in disease.

Funding Source: This work is supported by the Wellcome Trust and the ERC consolidator grant Relieve-IMD.

F-2007

NON-APOPTOTIC ROLE OF MCL-1 IN HUMAN PLURIPOTENT STEM CELLS

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Pluripotent stem cells maintain their unique properties of self-renewal and pluripotency through the rigorous expression of key transcriptional programs. Therefore, much effort has been devoted to the characterization of the transcription factors involved in maintaining stemness. However, much less is known about other fundamental traits of pluripotent stem cells, such as increased mitochondrial fragmentation and glycolytic profile in comparison to differentiated cells. We previously reported that human embryonic stem cells differentially regulate the apoptotic machinery by maintaining Bax in its active conformation at the Golgi rather than at the mitochondria. This allows stem cells to effectively minimize the risks associated with having pre-activated Bax and makes them acutely sensitive to DNA damage. We find that soon after differentiation, several apoptotic proteins are dynamically regulated. A key inhibitor of Bax is the Myeloid Cell Leukemia-1 (Mcl-1) protein. We find that, paradoxically, Mcl-1 levels decrease dramatically upon differentiation. We will show our findings that suggests that Mcl-1 regulates not only cell death, but also the ability of cells to self-renew and differentiate. Our data indicates that Mcl-1 may be an important mediator of mitochondrial dynamics in stem cells, and we will describe an unexpected non-apoptotic function for Mcl-1 in the maintenance of pluripotency.

F-2009

NOVEL FUNCTION OF CX30.3 IN REGULATING PLURIPOTENCY IN MOUSE EMBRYONIC STEM CELLS

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Based on the dynamic expression patterns, 19 connexin (Cx) isoforms in mouse embryonic stem cells could be classified into pluripotent state specific, differentiating stage specific, and non-specific Cxs. We focused on Cx30.3 as typical of the first category. Cx30.3 was pluripotent state-specific and upregulated by leukemia inhibitory factor (LIF), a specific cytokine that maintains the pluripotent state of ES cell, via a Jak signaling pathway. Cx30.3 protein was localized to both the cell membrane and cytosol. The dynamic movement of Cx30.3 in the cell membrane was suggested by the imaging analysis by means of overexpressed Cx30.3-EGFP fusion protein. The cytosolic portion was postulated to be a ready-to-use Cx pool. The Cx30.3 expression level in ES cell colonies dramatically decreased immediately after their separation into single cells. It was suggested that mRNA for Cx30.3 and Cx30.3 protein might be decomposed more rapidly than mRNA for Cx43 and Cx43 protein, respectively. These indicate possible involvement of Cx30.3 in the rapid formation and/or decomposition of gap junctions; implying a functional relay between Cx30.3 and other systems such as adhesion proteins. Cx30.3 transfected ES cells revealed statistically significant larger cell population compared with wild type. This indicates that Cx30.3 affected the cell cycle control system in undifferentiated ES cells.

F-2011

DEVELOPMENT OF LIVE-CELL FLUORESCENCE SCREENING PROBE FOR EARLY ISOLATION OF INDUCED PLURIPOTENT STEM CELL

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Regenerative medicine becomes the latest hot topic in medical research since the successful of induced pluripotent stem cell (iPSC) generation. However, the heterogeneity of the iPSCs and early detection of somatic cell reprograming still an obstacle for most stem cell researchers. The current method to identify iPSCs is detection of pluripotent marker genes expression either by RT-PCR or immunofluorescence staining. These methods are time consuming and sacrifice of valuable iPSCs. To solve this problem, our project aims at identifying a safe and fast, live-cell fluorescence staining probe that can be used to distinguish pluripotent stem cells from differentiated somatic cells. By screening the household novel fluorescently labelled small molecules library, we successfully identified a fluorescence dye that specially stained pluripotent stem cells. The dye named SW107 can selectively stain mouse embryonic stem cells (mESCs) and human iPSCs, while no signal detected from somatic cells including mouse tail and human skin fibroblasts. The stained stem cells can be also isolated by flow cytometry. The results show our SW107 dye can provide a more convenience way for iPSCs isolation which could facilitate regenerative medicine research.

F-2013

HUMAN NAÏVE PLURIPOTENT STEM CELLS WITH INCREASED FUNCTIONAL PLURIPOTENCY EXPRESS DEFINING MARKERS OF THE HUMAN PREIMPLANTATION EPIBLAST

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The derivation of naïve human pluripotent stem cells (N-hPSC) with improved functional pluripotency has broad impact for optimizing future hiPSC-based cellular therapies. We recently reported a novel LIF-3i (GSK3β, ERK and tankyrase inhibition) chemical reversion method that stably reverted hPSC to a naïve-like pluripotent state. LIF-3i N-hPSC possessed high clonal proliferation, MEK-ERK independence, bFGF signaling unresponsiveness, STAT3 phosphorylation, JAK-STAT3 and BMP4 signal dependence, increased naïve-specific transcript expression (e.g., DPPA3, NR5A2), upregulation of core pluripotency networks with decrease in lineage-primed gene circuits, whole genome transcriptomic clustering with both human preimplantation epiblast and mouse ESC, dominant distal OCT4 enhancer usage, global DNA CpG hypomethylation, increased 5hMC/5MC ratios, increased E-cadherin expression, and augmented expressions of cytoplasmic and nuclear activated β-catenin. Importantly, LIF-3i-reverted N-hPSC had significantly improved multi-lineage differentiation potency relative to their primed states. To further evaluate the phenotypic congruence of LIF-3i N-hPSC to human naïve preimplantation epiblast, we conducted a transcriptomic bioinformatics analysis and established a library of differentially expressed genes between conventional primed hPSC, LIF-3i

N-hPSC and recently published human preimplantation E3-E7 human epiblast cells. Extensive flow cytometry analysis revealed expression of a repertoire of naïve-specific human epiblast markers on LIF-3i N-hPSC (e.g., CD1d, CD3epsilon, CD5, CD7, CD14, CD24, CD44, CD46, CD55, CD90, CD99, CD131, CD151, CD164, CD180, CD248, CD325, HLA-ABC). Surface marker analysis of N-hPSC vs. primed hPSC lines revealed significantly increased expressions of human naïve epiblast-specific markers CD325, CD151, and CD44, and decreased levels of CD24, CD90, and HLA-ABC in LIF-3i N-hPSC (N=3). Moreover, LIF-3i N-hPSC demonstrated high protein levels of human E5-E6 epiblast-specific markers TRA-1-81, SSEA4, pSTAT3, NODAL, GDF3, and Axin-1, and decreased levels of pERk½ and LIN28. These findings confirmed previous bioinformatics findings demonstrating that LIF-3i N-hPSC possess key defining markers of the human naïve preimplantation epiblast.

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F-2015

INFLUENCE OF NANOTOPOGRAPHICAL FEATURES ON HUMAN PLURIPOTENT STEM CELL ADHESION AND GROWTH

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Human pluripotent stem cells (hPSCs) have tremendous potential to address a number of key issues in diverse fields such as regenerative medicine, disease modelling, toxicology, cellular therapy, tissue engineering, as well as contributing to a better understanding of human development and biology. One of the critical aspects of hPSC biology is to recapitulate the cellular niche that the cells would naturally be exposed to during development of the embryo. This facilitates the differentiation of hPSCs to a myriad of desired tissues. Traditionally PSCs have been cultured on two dimensional (2D) plastic due to its ease of use. However, development does not occur in 2D but rather three. The spatio-temporal arrangement of the cells, combined with topographical cues has great impact on differentiation and maturation of cells and tissues. One way to recapitulate this in vitro is by modulating the topography that hPSCs are exposed to, through the use of surfaces that present with nanotopographical features. Nanotopographies come in a variety of shapes and sizes from nano-pillars, -pits and -grooves. In this work, we utilized nano-pits with varying degrees of order/disorder in their arrangement. We demonstrate that hPSCs can be maintained on nanotopographies, while preserving pluripotency, differentiation potential, and both karyotypic and genomic stability. Importantly we have found that the nanotopographies support the adhesion and growth of human pluripotent stem cells using extremely low concentrations of extracellular matrices (ECM) such as recombinant human vitronectin and Laminin 511. These findings have the potential to improve hPSC culture by providing more physiologically relevant conditions for cell growth. Additionally, by reducing the amount of ECM required, the cost of expansion and maintainence can be significantly reduced.

F-2017

PRC2 SPECIFIES ECTODERM LINEAGES AND MAINTAINS PLURIPOTENCY IN PRIMED BUT NOT NAÏVE ESCS IN HUMAN AND MOUSE

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Polycomb Repressive Complex 2 (PRC2) is a evolutionarily conserved mechanism to mediate H3K27me3 and play a critical role in development and cancer. However, their role in cell fate decisions remain poorly understood. Here we report that ablation of PRC2 component genes in hESCs leads to an exit of pluripotency and spontaneous differentiation towards a meso-endoderm fate due to BMP activation. Consistently, EZH1-/- or EZH2-/- hESCs fail to differentiate into ectoderm. We further showed that PRC2 deficient mESCs also release BMPs, but maintain pluripotency until being converted into the primed state when they undergo similar spontaneous differentiation. Conversely, PRC2 is not required for pluripotency when hESCs are converted to a naïve state. Our studies reveal both lineage and pluripotent state specific role of PRC2 in cell fate decision.

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F-2019

CHARACTERIZATION OF COMMON MARMOSET ES CELLS IN THE NAÏVE PLURIPOTENT STATE

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Embryonic stem (ES) cells are pluripotent stem cell lines derived from inner cell mass (ICM) of blastocyst stage embryos. Distinct characteristics are expressed between primate and mouse ES cells. Currently, primate ES cells are considered as counterparts of mouse epiblast stem cells (EpiSCs), which is derived from the postimplantation embryo. The pluripotent state of mouse EpiSCs is termed primed state, distinguishing it from the naïve state of mouse ES cells. Primate ES cells have also been regarded as primed state pluripotent stem cells. However, although the derivation of naïve state pluripotency in primates has been reported previously, the naïve cells acquired have not been well characterized. Using ES cells of the common marmoset, a small non-human primate, we carried out the conversion from primed state to naïve state pluripotency. In this presentation, we report several characteristics of the resulting naïve marmoset ES cells. Briefly, these cells formed mouse ES cell-like dome-shaped colonies and maintained expression of pluripotency markers. Also, these cells showed similar gene expression pattern to mouse ES cells and ICM cells of the marmoset blastocyst. These phenotypes were lost when a JAK inhibitor was added to the culture, suggesting that the maintenance of this pluripotent state requires upregulation of the LIF/STAT3 signaling pathway. Furthermore, the naïve marmoset ES cells showed naïve-type energy metabolic profile. These properties closely resemble those of mouse ES cells, suggesting that the cells acquired a naïve pluripotency state. Finally, we found that the naïve common marmoset ES cells could contribute to the inner cell mass when they are introduced into the early embryos of both mice and marmosets. In the future, this technique would be useful for the production of genetically modified marmosets.

F-2021

ANALYSIS OF MICRORNA EXPRESSION IN RAT PLURIPOTENT STEM CELLS USING GENOME-WIDE SEQUENCING TECHNOLOGIES

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Rattus norvegicus is popular object for biomedical research studies such as disease modelling. For the purpose pluripotent stem cells (PSCs) such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are widely used. Rat ESCs were firstly derived in 2008 using special serum free conditions with the GSK3 and MEK1/2 inhibitors. Nevertheless pluripotency and self-renewal regulation in rat PSCs are poorly studied. Pluripotency regulatory network should be investigated thoroughly to improve generation and cultivation approaches of rat PSCs. MicroRNAs (miRNAs) are small noncoding RNAs, that are known to be an important part of the regulatory network. We employed Illumina sequencing to analyze miRNAs expression in the rat ESCs, iPSCs and embryonic fibroblasts (EFs), which were used for iPSCs generation. All PSCs lines were previously obtained in our lab and characterized using standard approaches. We performed bioinformatic analysis of raw data and found expression of 674 known miRNAs in all samples. We also found 404 novel miRNA candidates. Using differential expression analysis we have shown that 77 and 142 known miRNAs were upregulated in PSCs and EFs, respectively. Among novel miRNA candidates 10 were upregulated in PSCs and 13 – in EFs. We revealed significant differences in miRNAs expression between EFs and PSCs and no major difference between ESCs and iPSCs using hierarchical clustering and principal components analyzes. We searched validated target genes for differentially expressed known miRNAs, analyzed them using Gene Ontology, and found that the target genes were associated with transcription regulation, stem cell maintenance, cell proliferation, cell-cell adhesion, and regulation of cell differentiation categories. Using qRT-PCR we validated expression of three known miRNAs highly expressed in rat PSCs (miR-741-3p, miR-743a-3p, and miR-295-3p) and four novel miRNAs. We also analyzed their expression during PSCs differentiation and in rat adult organs, and suggested one novel

pluripotency associated miRNA based on expression pattern. Our work represents the basis for future studies of the pluripotency regulation in rat by miRNAs.

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F-2023

CLINICALLY COMPLIANT HUMAN PSC CULTURE CONDITIONS SUPPORT EFFICIENT CLONAL SURVIVAL AND RAPID SCALE-UP

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The lack of defined, xeno-free, easy and robust methods for efficient expansion of human pluripotent stem cells (PSCs) has hindered both the advancement of basic research and human cell therapy, much due to high experimental variation and poor quality cells with phenotypic and genetic changes. Laminin-521 (LN-521) is a protein naturally expressed by human PSCs and is a critical factor of the pluripotent stem cell niche. Laminins influence adhesion, differentiation, migration, phenotypic stability, anoikis resistance and functionality of all cells associated to it. LN-521 is a human and recombinant protein and can easily be used as a cell culture substrate. Human PSCs grow as a homogenous monolayer on LN-521, without any abnormal genetic aberrations and with maintained expression of pluripotency markers. Human PSCs on LN-521 expand twice as fast compared to other matrices and can be split 1:20 or up to 1:30 as single cells without the addition of ROCK inhibitor. Furthermore, LN-521 can be used as microcarrier coating for generating clinically relevant quantities of human PSCs thus offering a scalable and GMP-compatible bioprocessing platform. Moreover, true clonal culture, important for cell fate tracking, gene function analyses and editing, is possible by using LN-521. Human embryonic stem cell (hESC) lines can even be derived from a single blastomere under chemically defined and xeno-free condition on LN-521, thereby circumventing the ethical issues associated with hESCs. The simplicity and reliability of the culture procedure, the rapid cell amplification and the genetic stability of the cells make LN-521 a suitable as reagent in clinical trials for human PSC-based therapy. In conclusion, we show that LN-521 is an optimal matrix for human PSC culture due to its biological relevance that allows derivation, clonal cultivation, stable long-term pluripotent cell growth and scalability. The robust method allows minimum culture maintenance and standardized protocols, which can easily be adapted to automation platforms, making LN-521 a suitable reagent choice for human cell therapy trials.

F-2025

DEVELOPMENT OF AN IMPROVED FEEDER-FREE CULTURE SYSTEM FOR MOUSE PLURIPOTENT STEM CELLS

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Pluripotent stem cell models provide powerful tools for the researchers to study and model wide ranging biological questions, from fundamental developmental processes to translational medicine for understanding mechanisms of disease. Although recent advances in human induced pluripotent stem cell technology have rightfully garnered much excitement and use, mouse pluripotent/embryonic stem cells (mESC) continue to be useful and complementary tools, particularly for translating complex in-vitro studies assessing genetic modifications from the cellular level to whole animal model. Over decades, several reports have introduced methods for in vitro culture of mouse pluripotent stem cells including co culture with supporting fibroblast or small molecule based feeder independent culture. Optimum culture conditions need to support multiple applications including cell line derivation from recalcitrant strains, stable cell proliferation while maintaining pluripotency, and downstream in vivo applications like chimera formation. Here we present our development approach and results in generating a new feeder free mouse ESC culture medium. In assessing and optimizing mESC culture, we focused on 3 major attributes: (1) cell growth & colony morphology, (2) maintenance of pluripotency, and (3) ability to support downstream differentiation. Incorporation of multi-parametric Design of Experiment (DOE) approaches with robust cellular assays and automated imaging & analysis enables us to test multiple components in parallel and helped identify optimal conditions through iterative experimental rounds. Taken together this work highlights both (a) our design philosophy for culture media development- identify key functional endpoints, develop or incorporate robust, scalable assays, and test a wide array of components and workflow parameters; and (b) our results to date with this new system.

F-2027

IDENTIFICATION OF MIRNA-REGULATED PATHWAYS INVOLVED IN PLURIPOTENCY MAINTENANCE USING HIGH CONTENT ANALYSIS

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microRNAs (miRNAs) play an important role in stem cell's biology by binding to target mRNAs transcripts, inducing translation blockage and/or transcripts degradation. Upon differentiation of pluripotent cells, miRNAs can be induced or repressed, however, their specific roles are largely unexplored. We investigated the functional roles of a selected set of miRNAs in pluripotency and differentiation, using quantitative automated fluorescence microscopy (High Content Analysis; HCA). For this, we used NTera-2 (human embryonal carcinoma cells) and H1 (embryonic stem cells; ESC) as models. These cells were reverse-transfected (triplicate wells in 96-well plates) with thirty distinct miRs mimics (individually) or control molecules. Following 3-4 days of culture, cells were fixed, permeabilized and stained with Hoechst/CellMask Blue (nucleus/cytoplasm), anti-OCT4, anti-Cyclin B1 and imaged using an ImageXpress Micro HCA System. CellProfiler was used to quantify several morphometric parameters and intensity measurements of OCT4 and CYCB1 in nuclear and cytoplasmic compartments. Quantified parameters were used to generate miR-specific multiparametric phenotypic profiles (using KNIME) that were submitted to unsupervised clustering, allowing the identification of miRs with similar phenotypic effects. By identifying predicted targets (TargetScan) shared by the clustered miRs, and by submitting these target sets to enrichment functional analyses (DAVID), we were able to identify pathways and processes involved in the control of pluripotency. As an example, members of the miR-17 family (miR-302a, b, c and d-3p), miR-19a-3p and miR-20a-5p were all found to promote pluripotency features (OCT4 and CYCB1 intensity) in NTera2 and H1 ESC. Importantly, by identifying shared targets, like Cyclin D1 and D2, MBD2a and HDAC4, previously shown to mediate miR-302 positive effects in pluripotency, we show that miR-19a and miR-20a are also able to promote pluripotency trough this specific shared regulatory mechanism.

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PLURIPOTENT STEM CELL DIFFERENTIATION

F-2031

SELF-RENEWING EARLY LUNG EPITHELIAL PROGENITOR CELLS DIFFERENTIATED FROM HUMAN EMBRYONIC STEM CELLS

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Under healthy conditions, the lung airway epithelium undergoes a slow but constant renewal with turnover occurring every 30-50 days. In airway diseases such as asthma, chronic obstructive pulmonary disease, obliterative bronchiolitis, and cystic fibrosis, the airway epithelium is damaged and requires repair. In these cases the repair capacity provided by endogenous lung epithelial progenitor cells is often insufficient. Currently, it is a challenge to obtain sufficient numbers of primary lung epithelial progenitor cells that could possibly be used for therapy and/or tissue engineering applications. Here we describe the cells differentiated in vitro from human embryonic stem cells (hESC) that can be propagated for long-term in culture and most likely represent equivalent of early lung progenitors (ELP) occurring in development. Briefly, the protocol to derive these in vitro growing ELP includes initially driving the cells towards endodermal lineage by 5-day exposure to Activin A, then pushing the cells towards anterior foregut endoderm by serum-free conditions for 2 days, followed by passaging the cells in low density in low-serum media supplemented with FGF2 and EGF. We have shown that these cells (ELP) can be maintained in culture for a minimum of 65 passages (also including freezing/thawing step) without losing their key characteristics. ELP maintain their population doubling time at an average of 26.5 hrs and the activity of their telomerase holds at about 50% of that typical for undifferentiated hESC. ELP express high levels of anterior foregut marker SOX2 (also typical for self-renewing cells), marker of definitive endoderm SOX17, and marker of early lung epithelial lineage, thyroid

transcription factor-1 (TTF1). As found by transmission EM, ELP also possess morphological features of cells differentiating towards airway epithelia, multivesicular and lamellar bodies. When induced (in 2D format; by FGF7, FGF10, and EGF) to terminally differentiate, ELP increase levels of FOXJ1 (ciliated cells), pro-surfactant protein B (alveolar epithelial cells), Club cell specific protein (Club cells), aquaporin A (type I pneumocytes), and surfactant proteins A and C (type I pneumocytes and Club cells). Under 3D conditions, differentiating ALP then develop morphologies of alveolar- and airway-like structures.

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F-2033

PROGRAMMING CELL FATE USING THE HUMAN TFOME

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The ability to produce any human cell in a robust and facile manner would advance the development realistic microtissues and organoids. To expand the breadth and access of in vitro cell types, we conducted large-scale cell fate engineering from human induced pluripotent stem cells (hiPSCs). First, we created a comprehensive human transcription factor (TF) expression library (the "human TFome") to systematically screen TFs that differentiate human stem cells. Then, we mapped transcription factor-cell fate relationships by RNA sequencing. From these approaches, we identified 78 single TFs that program pluripotent stem cells into differentiated cell types derived from multiple tissues. We achieved potent (>80%) cellular programming without the addition of growth factors, mechanical processes or purifications by maximizing TF expression and selecting potent TF isoforms. Engineered cell fates include electrically active neurons, lumen-forming endothelial cells and myoblasts. Large-scale engineering of human cell fates could pave the way towards the production of many human cell types in vitro.

F-2035

SUBTYPE-SPECIFIC BIAS INTRODUCED BY PRONEURAL GENES DURING NEURONAL PROGRAMMING

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The vertebrate central nervous system consists of diverse neuronal cell types to generate functional circuits. How the different phenotypic features underlying this diversity are established during development remain unclear and it poses a significant challenge in current efforts to generate clinically relevant neurons. Ascl1 and Ngn2 are the two main basic helix-loop-helix proneural transcription factors (TFs) in vertebrates, and thus are frequently used in direct programming of neurons from pluripotent and differentiated cells. Even though both TFs initiate generic neuronal differentiation program, they have divergent functions in the specification of subtype-specific neuronal features, which is largely neglected in the choice of TF combinations to program neurons. Therefore, the goal of this study is to understand the mechanism of subtype-specific bias introduced by Ascl1 and Ngn2 during neuronal programming from mouse embryonic stem cells (mESCs). To that end, we generated inducible mESC lines expressing Ascl1 and Ngn2. Gene expression analysis by RNA-seq suggests that even though both TFs activate a generic neuronal expression profile, each TF induces expression of distinct subtype-specific genes. To test whether this subtype-specific bias in gene expression is due to their differences in the genome-wide binding pattern, we analyzed the initial binding events of Ascl1 and Ngn2 12 hours after induction by ChIP-seq. We found that binding of Ascl1 and Ngn2 largely differs, sharing only 10% of the sites. Moreover, this binding difference is not dominated by the ability of each factor to bind to preexisting inaccessible regulatory regions. Then, we asked whether the difference in the binding pattern stems from a sequence-specific bias at Ascl1 and Ngn2 differentially bound sites. We searched for enrichment of specific 6-mer sequences in a 150 bp window around Ascl1 and Ngn2 differential peaks and counted the fraction of peaks with each of the specific 6-mer sequence. We found that the specific 6-mer sequence enriched at Ascl1 and Ngn2 sites differ by only one base pair: Ascl1-bound sites have a distinct E-box motif as CACCTG, Ngn2-bound sites have CATCTG. Therefore, our working model suggests that DNA-binding specificity is what drives the subtype-specific bias in neuronal programming by Ascl1 and Ngn2.

F-2037

PATH OF LEAST RESISTANCE: MODELING THE BBB OF PATIENTS WITH X-ALD USING DIRECTED DIFFERENTIATION OF HUMAN IPSCS INTO BRAIN MICROVASCULAR ENDOTHELIAL CELLS (BMECS)

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X-linked adrenoleukodystrophy (X-ALD) is an inherited metabolic storage disorder caused by mutations in the ABCD1 gene. ABCD1 is a peroxisomal transporter protein responsible for transporting very long-chain fatty acids (VLCFAs) from the cytosol into the peroxisome for subsequent beta-oxidation. Accumulation of VLCFAs causes demyelination in the long tracts of the spinal cord and the progressive axonopathy seen in adrenomyeloneuropathy (AMN). A more severe phenotype that affects a subset of males early in life is childhood cerebral ALD (ccALD). ccALD is characterized by rapid inflammatory demyelination in the brain and death within a few years. The molecular mechanisms responsible for the onset and progression of ccALD remain poorly understood. The rapid demyelination characteristic of ccALD is first detectable by MRI as gadolinium enhancement at the center of the corpus callosum. The initial breakdown is thought to be mediated by immune cells (specifically T-cells) that translocate from the blood into the brain. Thus, we decided to investigate whether the integrity of the BBB of patients with ccALD is decreased compared to wild-type (WT) controls. Using a previously published method for the directed differentiation of human pluripotent stem cells into blood brain barrier (BBB) endothelium, we directed the differentiation of iPSCs into BMECs using iPSCs from three patients with ccALD and three WT controls. Immunofluorescence and RT-PCR demonstrate that both patient and control BMECs express the requisite endothelial markers PECAM-1, VE-Cadherin, and von Willebrand factor, the tight junction markers Claudin-5 and Occludin, and the BBB markers P-gp and GLUT-1. To assess barrier integrity across these cells, we used trans-endothelial electrical resistance (TEER). Our results show that BMECs from patients with ccALD have significantly decreased TEER compared to WT controls. We are in the process of testing the passive transport between cells using a sodium fluorescein permeability assay and the P-gp efflux potential of the cells using a Rhodamine 123 accumulation assay as well as performing RNA-seq of the derived BMECs to discover novel pathways contributing to the ccALD disease phenotype and the screening small molecules or drugs to target these pathways to increase the BBB integrity of patients with ccALD.

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F-2039

MYC CONTROLS PLURIPOTENT STEM CELL FATE DECISIONS THROUGH REGULATION OF METABOLIC FLUX

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As human pluripotent stem cells (hPSCs) exit pluripotency they are thought to switch from aerobic glycolytic to a mode of energy generation more dependent on oxidative phosphorylation. However we show, through Seahorse metabolic analysis and C13-labeling assays, that metabolic switching occurs only during early mesoderm and endoderm differentiation, and that elevated glycolytic flux is maintained and essential during early ectoderm specification. Inhibition of glycolysis within hPSCs results in the loss of pluripotency markers and up-regulation of endoderm and mesoderm genes, but early ectoderm genes are not induced. Since aerobic glycolysis is retained in early ectoderm formation, metabolic switching is therefore not an obligatory event required for exit from pluripotency. MYC/MYCN binds to the promoters of key metabolic genes, such as LDHA, HK1 and PKM1/2, whose elevated expression establishes aerobic glycolysis in hPSCs. Elevated MYC/MYCN activity is required for maintenance of aerobic glycolysis, and therefore self-renewal, within hPSCs. In endoderm and mesoderm, decreased MYC/MYCN transcriptional activity coincides with metabolic switching, but aerobic glycolysis can be re-established by ectopically restoring MYC activity. Knockdown of MYC/MYCN, via shRNAs, within hPSCs results in the loss of aerobic glycolysis and induces a spontaneous differentiation to endoderm and mesoderm but not ectoderm, similar to glycolytic inhibition. MYC activity is therefore necessary and sufficient for metabolic switching. In nascent ectoderm, sustained MYCN activity maintains the transcription of 'switch' genes that are rate-limiting for metabolic activity and lineage commitment. This study identifies MYC and MYCN as developmental regulators that couple metabolism to pluripotency and cell fate determination.

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F-2041

HUMAN EMBRYONIC STEM CELL-DERIVED NEURAL STEM CELLS: ROLE OF MICRORNA AND CELL CYCLE IN SHAPING STEM CELL IDENTITY

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Regeneration and replacement of neurons and glia that undergo cell death are the main goals of stem cell-based therapies. Clinically-relevant methods for generation of neural stem cells (NSCs) from human embryonic stem cells (hESCs) only begin to emerge. We have previously derived such self-renewing NSC lines with the ability to differentiate into functional neurons and glia in vivo. Importantly no tumor formation was noted, therefore studying self-renewing mechanisms of NSCs with "non-tumorigenic nature" might shed a light on deregulated proliferation mechanisms often found in CNS tumors. Thus, the aim of the present study was to define molecular mechanisms responsible for the maintenance of unlimited self-renewal of NSCs. Since self-renewal and differentiation has been previously connected with the regulation of cell cycle partially via microRNA in hESCs, we hypothesize that differentiation-associated miRNAs contribute to cell cycle regulation in self-renewing NSCs as well. We initiated our experiments by analyzing high throughput microRNA expression data in undifferentiated hESCs, self-renewing NSCs, and non-self-renewing neural progenitor cells (NPCs). Results revealed several candidate microRNAs which are specifically highly upregulated in NSCs, but not in NPCs. Bioinformatic predictions suggest that these microRNAs regulate cell cycle progression and might thus contribute to maintenance of self-renewing phenotype in NSCs. Furthermore, our mRNA sequencing analysis of the same cell types revealed 1) candidate transcription factors directly linked to cell-cycle regulating microRNAs; and 2) other regulatory proteins with putative cell-cycle functions specifically in NSCs. Functional studies are ongoing to reveal underlying regulatory pathways for self-renewal of hESC-derived NSCs.

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F-2043

REGENERATIVE PROPERTIES OF IPS CELL-DERIVED SKELETAL MUSCLE STEM CELLS FROM PATIENTS WITH FIBRODYSPLASIA OSSIFICANS PROGRESSIVA

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Skeletal muscle and bone are critical components of the musculoskeletal system, yet our understanding of how these two tissues interact to cause disease pathology is still rudimentary. One particularly dramatic response to muscle injury is the development of heterotopic ossification (HO), a condition where bone inappropriately forms in a soft tissue. For unclear reasons, HO most commonly occurs in skeletal muscle. Patients with mutations in the Activin A Type I receptor (ACVR1), a bone morphogenetic protein (BMP) receptor, develop the debilitating disease fibrodysplasia ossificans progressiva (FOP) with massive heterotopic ossification principally within their skeletal muscles. Our overall hypothesis is that activated BMP signaling caused by the ACVR1 R206H mutation incites inappropriate activation of human muscle stem cells. The resulting loss of muscle cell fate then leads to HO in skeletal muscles. Since it is often difficult or impossible to obtain large amounts of human satellite cells from live tissue donors, human iPS cell-derived muscle stem cells would provide an attractive source for our studies. We successfully optimized recently developed protocols to create muscle stem cells from a collection of 3 characterized FOP and 3 control iPS cell lines to create Pax7+ muscle stem cells with high efficiency. We developed a sorting strategy based on the expression of CD56 and CD29, two markers expressed by Pax7+ cells, to further purify the Pax7+ muscle stem cells. Our iPS cell-derived muscle stem cells expressed similar levels of Pax7 to primary

satellite cells and low levels of Myogenin, consistent with a skeletal muscle precursor. We are now using an injury mouse model of the tibialis anterior muscle to investigate the regenerative potential of our iPS cell-derived muscle stem cells. Our preliminary results show that control and FOP cells can engraft and contribute to the formation of new fibers after transplantation. This study provides a new way to investigate increased BMP signaling in human muscle stem cells using a unique humanized in vivo model. Our ability to create and purify a population of Pax7+ cells from human iPS cells derived from patients will be useful to identify new therapeutic targets for bone and muscle diseases, and will improve our understanding of how these two key skeletal tissues interact.

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F-2045

TRANSCRIPTION FACTOR SOX21 PLAYS AN IMPORTANT ROLE IN THE FOREBRAIN SPECIFICATION DURING NEURAL DIFFERENTIATION OF HUMAN ESCS

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Neural induction and patterning constitute the initial step in the development of the nervous system. However, the molecular circuitry underlying these important processes is still poorly understood. Here, we report that transcription factor SOX21 inhibits Wnt signaling to promote the forebrain specification. We begin with profiling dynamic transcriptomes during neural differentiation from human embryonic stem cells (hESCs) using a dual SMAD inhibition protocol to identify specific gene signatures at different stages along with neural differentiation process. At the early stages, differentiating cells reshape metabolic networks and acquire the ectodermal identity, whereas cells at the late stages switch cell cycle processes and the cytoskeleton architecture to differentiate into neuronal lineages. Specifically, we find that the expression of SOX21 is repressed by OCT4, being silenced in undifferentiated hESCs. However, it displays transient upregulation at the early stage of hESC neural differentiation. Overexpression of SOX21 in hESCs disrupts self-renewal and leads to neural differentiation. Deletion of SOX21 using the CRISPR/Cas9 approach does not affect hESC self-renewal, induces apoptosis during neural differentiation. Moreover, we find that SOX21 promotes the forebrain specification at the early stage of neural differentiation. Mechanistically, SOX21 binds to the regulatory region of WNT8B, an activator of the canonical Wnt-beta-catenin signaling pathway, and represses its expression. It could promote the forebrain specification through repressing Wnt signaling during early neural differentiation. Collectively, this study reports highly dynamic transcriptomes during hESC neural differentiation and uncovers a novel transcription factor that is activated during human neuroectoderm development and promotes the irreversible fate specification of human pluripotent cells toward the forebrain lineage.

F-2047

ENHANCED IN-VITRO DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELL EMBRYOID BODIES INTO HEPATOCYTE-LIKE CELLS BY INTERLACING HUMAN ENDOTHELIAL CELLS

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Differentiation of human induced pluripotent stem cells (hiPSC) into functional hepatocyte-like cells (HLC) has applications in cell therapy, bioengineered organs and drug testing. This study used a novel suspension-based embryoid body (EB) differentiation protocol for the generation of iPSC-derived HLC and testing the effect of co-culture with human endothelial cells (hEC) on improving function after transplantation in an animal model of acute liver failure. EB with hiPSC only (hiPSC-EB) and containing hiPSC mixed with hEC (hiPSC+EC-EB) were generated using agarose molds. hiPSC-EB and hiPSC+EC-EB were differentiated into HLC using a four-stage suspension culture-based differentiation protocol. Both hiPSC-EB-HLC and hiPSC+EC-EB-HLC were screened for several hepatic functionalities and markers in vitro and transplanted into the spleens of athymic rats with d-galactosamine induced acute liver failure. The differentiation of hiPSC-EB and hiPSC+EC-EB into HLC was confirmed by the presence of gene expression and immunofluorescence for several hepatocyte markers. hiPSC+EC-EB-HLC showed increased amount of Albumin secretion in vitro compared to the hiPSC-EB-HLC. hiPSC+EC-EB-HLC displayed higher secretion of Fibrinogen and Urea as compared to the hiPSC-EB-HLC. Hepatocyte function in vitro, such as Acetylated low-density lipoprotein (DiI-ac-LDL) uptake, Indocyanine green (ICG - Cardiogreen) absorption and release, Glycogen storage, and cytoplasmic accumulation

of neutral triglycerides and lipids were comparable between the two conditions. Induction of several cytochromes P450 (CYP450) using different inducers showed a significant increase in the activity of all the CYP450 tested from baseline for the hiPSC+EC-EB-HLC in comparison to the hiPSC-EB-HLC. Differentiated cells displayed gene expression of all the intrinsic and extrinsic coagulation factors, showing the ability of both HLC and hEC to function as one organoid unit. Transplantation of hiPSC+EC-EB-HLC was associated with sustained rat serum human albumin at 14 days after transplant as compared to 3 days after transplantation among the hiPSC-EB-HLC group. Differentiation of hEB into functional hiPSC-derived HLC and incorporation of hEC with hiPSC provides more sustained hepatocyte function in vivo after transplantation.

F-2049

IDENTIFY AND CHARACTERIZE CELL SURFACE PROTEIN MARKERS OF MATURE MOTOR NEURONS TO ENABLE LIVE MOTOR NEURON IDENTIFICATION, REAL TIME TRACKING OF PHYSIOLOGY AND CYTOLOGICAL PURIFICATION OF MOTOR NEURONS

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iPSC's are powerful cellular models of human disease. In Amyotrophic Lateral Sclerosis (ALS), cortical and spinal motor neurons are the cells that degenerate in patients and thus are the primary cell types being studied by many research groups interested in revealing the pathology of ALS. A major challenge facing the field of iPSC modeling of motor neuron degeneration in ALS, is the inability to precisely identify bona fide motor neurons in cultures differentiated from iPSCs. While recent advancements in genomic profiling and cytological imaging technologies enables single cell resolution of motor neuron identity and physiology, these techniques necessitate the destruction of motor neuron viability. In other words, the identification of motor neurons occurs after motor neurons are killed; there is no method to retain live cultures of motor neurons after their identification. The goal of this study is to identify and characterize cell surface protein markers of mature motor neurons, either in induced pluripotent stem cell (iPSC)-derived cultures or in vitro cortical and spinal cord histological samples. This would enable live motor neuron identification, real-time tracking of physiology, and cytological purification of motor neurons. We have integrated surfacesome, proteomics, and single cell RNA-seq data to identify transmembrane proteins selectively expressed in adult motor neurons.

Funding Source: CIRM

F-2051

HUMAN IPSC-DERIVED PATIENT SPECIFIC TISSUE GRAFTS TO ACTIVATE A REPAIR PHENOTYPE FOR DIABETIC FOOT ULCERS

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Diabetic foot ulcers (DFUs) are non-healing wounds which are a debilitating complication of diabetes that can lead to lower extremity amputation in patients. There is a large unmet need to find novel and efficient therapies to treat DFUs. To address this, we have developed a new tissue engineering approach by fabricating patient-specific, 3D FIB-iPSC-derived tissue grafts (GRiPS) that incorporate bioactive fibroblasts that were differentiated from induced pluripotent stem cells (FIB-iPSCs). FIB-iPSCs were derived from reprogrammed human primary diabetic foot fibroblasts (DFFs), diabetic foot ulcer fibroblasts (DFUFs) and primary normal foot fibroblasts (NFFs), which were then differentiated into fibroblasts (iDFUFs, iDFFs, iNFFs). FIB-iPSCs assembled an extracellular matrix (ECM) when grown using in vitro, 3D tissue engineering principles generating 3D GRiPS. We then evaluated capacity of these GRiPS to activate a pro-repair phenotype . We performed side-by-side comparisons to establish the in vitro and in vivo phenotypes of these GRiPS. GRiPS generated from iDFUFs, iDFFs and iNFFs show a unique acellular, non-fibrillar morphology when compared to tissues constructed from primary DFUFs, DFFs and NFFs. GRiPS were characterized by: (1) decreased levels of major wound healing collagens Type I and Type III; (2) increased levels of fibronectin and Type IV collagen; (3) increased amounts of sulfated glycosaminoglycans; and (4) decreased amounts of hyaluronic acid when compared to 3D scaffolds fabricated from their primary cell counterparts. All types of iPSC-derived fibroblasts demonstrated these phenotypic features suggesting "phenotypic convergence" after reprogramming and differentiation. When GRiPS were transplanted to mice,

tissue grafts persisted, underwent limited remodeling and showed increased wound closure in diabetic mice, suggesting the utility of FIB-iPSC-derived ECM for DFU repair. The development of these new tissue engineered platforms from iPSC-derived fibroblasts can now be used to impact compromised wound repair where tissue grafts can provide a pre-formed, structural ECM and viable growth factor-producing cells needed to stimulate healing of chronic wounds.

Funding Source: NIH Grant No. R01 DK98055-06 (J.A.G.)

F-2053

IN VITRO MODELS OF GJB2-RELATED HEARING LOSS RECAPITULATE THE FUNCTIONAL GAP JUNCTION PLAQUE FORMATION CHARACTERISTIC OF DEVELOPING COCHLEA

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Mutation of the Gap Junction Beta 2 gene (GJB2) is the most frequent cause of hereditary deafness worldwide and accounts for up to 50% of non-syndromic sensorineural hearing loss cases in some populations. GJB2 encodes connexin (CX) 26, a component in cochlear gap junction. We have demonstrated that the drastic disruption of gap junction plaque (GJP) macromolecular complex composed of CX26 and CX30 are critical pathogenesis starting before hearing onset. Therefore, cochlear CX26-gap junction plaque (GJP)-forming cells such as cochlear supporting cells are thought to be the most important therapeutic target for the treatment of hereditary deafness. The differentiation of pluripotent stem cells such as induced pluripotent stem (iPS) cells into cochlear CX26-GJP-forming cells had not been reported. To develop the effective therapy for GJB2 associated hearing loss, restoration of GJP macromolecular complex using iPS cells are expected to rescue the hearing function of GJB2 related hearing loss. Here, we detail the development of a novel strategy to differentiate induced pluripotent stem cells into functional CX26-GJP-forming cells that exhibit spontaneous ATP- and hemichannel-mediated Ca2+ transients typical of the developing cochlea. Furthermore, these cells from CX26-deficient mice recapitulated the drastic disruption of GJPs, the primary pathology of GJB2-related hearing loss. These in vitro models should be useful for establishing inner-ear cell therapies and drug screening that target GJB2-related hearing loss.

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F-2055

BETA CELL REPLACEMENT IN MICE USING HUMAN TYPE 1 DIABETES NUCLEAR TRANSFER EMBRYONIC STEM CELLS

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Beta cells derived from pluripotent stem cells hold great promise for autologous cell replacement therapy. Here we examine the ability of nuclear transfer embryonic stem cells derived from a T1D patient (1018-NT-ES) to differentiate into beta cells (1018-NT-beta) and to rescue diabetic mice. We found that 1018-NT-ES cells differentiate in vitro with an average efficiency of 55% into C-peptide-positive cells, expressing canonical markers of mature beta cells, including MAFA and NKX6.1. 1018-NT-beta cells show increased insulin secretion in response to various secretagogues, and increased cytosolic calcium in response to glucose. Important differences relative to naturally occurring pancreatic beta cells are present, including a modest incremental response to glucose, less insulin content, and reduced insulin processing. Upon transplantation in immunodeficient mice, 1018-NT-beta cells form vascularized islet-like structures that contain MAFA-positive C-peptide-positive cells and interspersed glucagon-positive cells. These beta cells show a normal proinsulin to insulin ratio, and adapt insulin secretion to ambient metabolite status of the mouse. Importantly, 1018-NT-beta cells were able to maintain normal blood glucose levels after ablation of the mouse's endogenous beta cells using streptozotocin. Cystic structures, but no teratomas, were observed in 1018-NT-beta cell grafts. Two isogenic induced pluripotent stem cell lines (1018-iPSC A and 1018-iPSC E) derived from the same patient by RNA reprogramming showed greater variability in beta cell differentiation, with fewer C-peptide-positive cells (10% and 45%) developing in vitro. Similar differences were observed between nuclear transfer embryonic stem cells and induced pluripotent stem cells from a healthy control donor (BJ-NT-ES 5 and BJ-NT-ES 6 vs BJ-iPSC M and BJ-iPSC O) towards beta cell differentiation. These results indicate that differentiation outcomes are cell line dependent, and though induced pluripotent stem cell lines are molecularly similar, full differentiation competence may be more common in

embryonic stem cell lines. These results provide the first proof of principle of therapeutic cloning combined with autologous cell replacement.

F-2057

A GENETIC SCREEN IN HAPLOID ES CELLS UNCOVERS A ROLE FOR RNA BINDING PROTEINS IN THE EXIT FROM PLURIPOTENCY

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Cell fate changes during embryonic development require the irreversible rewiring of transcription factor networks and epigenetic frameworks to establish and stabilise lineage identity. Embryonic cell fate decisions can be modelled in culture in embryonic stem (ES) cells. The transcription factor networks and epigenetic constraints that maintain ES cell identity are well described. Much less understood is how the highly recursive transcription factor network that defines pluripotency is dismantled at the exit from pluripotency. Haploid ES cells provide a platform for unbiased random mutagenesis based screens in mammalian cells. By combining powerful improved mutagenesis methodologies and efficient protocols for transposon integration mapping, we have driven a screen to identify key players in the exit from pluripotency to saturation. In total, we have screened approximately 1x10^6 mutant ES cell clones in 35 medium scale screens and have defined a cohort of 300 high-confidence candidate genes. In addition to the expected transcription factors and chromatin modifiers, the list of candidate genes contained several genes that are involved in the regulation of RNA homeostasis. Specifically, several components of the nonsense mediated mRNA decay cascade were found among the top candidates. We are now testing the hypothesis that NMD is used to shape transcriptomes in a way to facilitate rapid cell fate changes that accompany ES cell differentiation. Indeed, genetic deficiency for NMD factors results in differentiation delays without interfering with self-renewal. Furthermore, also downstream lineage decisions appear to be affected in NMD deficient ES cells. During self-renewal pluripotency transcription factors (including myc), are not upregulated. Therefore, the relevant targets of NMD regulation remain to be discovered. We are analysing the impact of NMD deficiency on global RNA levels in steady state ES cells and at the exit from pluripotency, as well as by measuring the impact of NMD deficiency on mRNA half-lives during differentiation. We have also implemented a PAR-CliP protocol to map the direct targets of NMD activity. By intersecting these methodologies bioinformatically, we aim to delineate relevant primary targets of NMD that are causative for the observed differentiation defects.

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F-2059

LUNG CELL FATE IS REGULATED BY SIGNALLING BETWEEN ENDOTHELIAL CELLS AND LUNG PROGENITORS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Bioartificial organ replacement therapy, incorporating induced pluripotent stem cell (iPSC) derivatives for patient-specific tissue repair, is a novel regenerative strategy to address the growing burden of severe lung diseases. Presently, diverse methodologies exist for specifying proximal airway compared to distal epithelium differentiation from PSCs. Since epithelial-endothelial cell interactions are crucial for the formation of the alveolar-capillary barrier in lung homeostasis, this study investigated the role of endothelial cells (ECs) in influencing cell fate choice of de novo lung progenitors derived from hiPSCs. HiPSCs were cultured on Matrigel during their differentiation into ECs or lung progenitors. EC differentiation involved treatment of hiPSCs with BIO and VEGF, isolation of VE-cadherin+ cells and an expansion phase during which conditioned medium (EC-CM) was collected. Lung embryogenesis was recapitulated via the definitive endoderm followed by CXCR4+ selection. These cells were cultured with a combination of factors (i.e. hedgehog, bone morphogenetic proteins, fibroblast growth factors) to yield lung progenitors. Lung progenitors were then cultured in 50% EC-CM with daily media changes for 14 days. qRT-PCR analyses revealed significant (#$p < 0.01$) downregulation in pluripotency genes (NANOG, OCT4, SOX2) upon differentiation into ECs or lung progenitors. Flow cytometric verification of CD34 and PECAM-1 expression signified successful EC differentiation. TTF1 and SOX9 were highly upregulated in the lung progenitor pool but not in prior stages (*$p < 0.05$). Interestingly, lung cells cultured in EC-CM were biased towards distal and not proximal airway patterning as evidenced by augmented expression of mature alveolar pneumocyte markers (AQP5, SPB, SPC); concomitant with reduced gene expressions associated with ciliated (FOXJ1) or goblet cells (MUC5AC). Together, our results illustrate the essential role ECs play not only in gas exchange, but also in defining lung cell fate during early lung organogenesis through paracrine mechanisms. This finding is relevant for the future establishment of in vitro lung models for disease modelling (i.e. organoid cultures) that endeavor to improve the efficiency of

deriving a predominantly alveolar as opposed to a proximal epithelium.

F-2061

ESTABLISHING AN ORGANOID SYSTEM TO MODEL GASTRULATION IN VITRO USING MOUSE PLURIPOTENT STEM CELLS

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Gastrulation is a paradigm for coordinated cell fate specification whereby the germ layers are specified and patterned providing a blueprint for the adult body. However, research on human embryonic material at this stage is not possible and the implanted mouse embryo is relatively inaccessible for long-term experiments hence little is known about the dynamics of these events. Recently, an in vitro micropatterning system (MPS) using human embryonic stem cells (ESCs) demonstrated both differentiation and spatial patterning of germ layers, similar to events occurring in vivo during gastrulation. To determine whether this system accurately recapitulates mammalian embryonic development, I have established a comparable system using mouse pluripotent stem cells. I have quantitatively analyzed marker expression throughout MPS differentiation and compared this directly to protein expression in the mouse embryo throughout gastrulation. I will take advantage of fluorescent reporter lines and genetic tools to analyze these events in real time at a single cell level. Currently I am manipulating signaling pathways individually and in combination to map the role and temporal requirements of particular cytokines for differentiation and patterning. This system offers a high-throughput, quantitative and easily manipulated tool to model gastrulation-like events in vitro.

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F-2063

COMBINED DELIVERY OF PERICYTE-LIKE INDUCED PLURIPOTENT STEM CELL-DERIVED SMOOTH MUSCLE CELLS AND ENDOTHELIAL CELLS ENHANCED REVASCULARIZATION OF DECELLULARIZED LUNG SCAFFOLDS

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Bioartificial lungs represent a novel alternative for organ transplantation; however, inadequate revascularization has limited in vivo function and survival of recellularized lung-scaffolds. Smooth muscle cells (SMCs), including both iPSC-derived SMCs (iSMCs) and endogenous pulmonary artery-derived SMC (PASMC) were co-cultured with GFP-HUVECs across a range of ratios. Analysis of branching-length and node numbers suggest an optimal SMC:HUVEC ratio of 3:1. Immunoflourescence revealed spontaneous HUVEC-iSMC alignment, recapitulating in vivo vessel anatomy, whereby vessel-stabilizing pericytes afford structural support. Accordingly, flow cytometric data demonstrated >95% expression of pericyte markers CD146 and PDGFRb in iSMCs but not PASMC (< 15%). EC-alone networks collapsed < 16Hrs; however, network persistence lasted < 36hrs with HUVEC-PASMCs and >72hrs for HUVEC-iSMCs. Vessel stabilization is also a function of reciprocal EC-SMC gene-regulation. To study this interaction, HUVEC-SMC co-cultures was performed for 24-72Hrs, prior to gene analysis. Relative to HUVEC alone, co-cultured HUVECs increase expression of EC, angiogenic and matrix genes as time progressed. To gain further mechanistic insights underlining network persistence disparity conferred by HUVEC-PASMC and HUVEC-iSMC, 72Hr time-point samples were further subjected to angiogenesis-focused PCR microarray. A significant increase in pro-angiogenic genes and reduction in angiogenic-inhibitory genes in HUVEC-iSMC compared to HUVEC-PASMC co-cultures was noted. Q-PCR and immunostaining also showed marked elevation in extracellular matrix proteins in co-cultured HUVECs. Subsequent histological studies of decellularized rat lung scaffold seeded with iSMCs and iECs (3:1 ratio), demonstrated revascularization as rapidly at D3 post-seeding. Importantly, cells continue to survive and engraft within the scaffold till at least D5 post-seeding. Collectively, we demonstrate that enhancement of revascularization is mediated by structural support and EC-SMC regulation of angiogenic, endothelial and extracellular matrix genes. Mechanistic insights underlying this improved revascularization strategy provide viable biotherapeutic targets employable across a range of organ recellularization endeavours.

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F-2065

ASSESSING THE ROLE OF A NOVEL LONG NON-CODING RNA, PLATR4, IN LINEAGE COMMITMENT

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The mammalian genome encodes a huge repertoire of non-coding RNAs. Long non-coding RNAs (lncRNAs), >200 nucleotides in length, are an important class of ncRNAs with the potential to impact gene regulation. LncRNAs are differentially expressed in a development-

specific manner across tissues, suggesting regulatory roles in cell fate decision and differentiation. Here we have identified the functional role of a novel embryonic stem cell (ESC)-specific lncRNA, Platr4 (pluripotency-associated transcript 4) in lineage commitment and differentiation processes. Platr4 is a 986 nucleotide poly(A)+ transcript comprising two exons consistent with our RNA-seq and Northern analysis in ESCs. Cellular fractionation of mouse ESCs indicates that Platr4 is mainly present in the nuclear fraction and associated with chromatin. Using the CRISPR/Cas9 genome-editing system we have generated a mouse ESC line (AB2.2) with deletion of the Platr4 promoter resulting in a significant depletion (homozygous deletion, Platr4-knockout) of the Platr4 transcript (up to 99%) as measured by qRT-PCR and single-molecule RNA-FISH analysis. Deletion of Platr4 in ESCs did not affect colony morphology, cell cycle kinetics or pluripotency. In contrast, we identified abnormalities in the spontaneous contraction of embryoid bodies (EBs) in Platr4-knockout cells, compared to control cells, during the differentiation process. In control cells, 27% of EBs beat at day 12 compared to 2% in Platr4-depleted cells. Consistent with this data the expression of cardiac Troponin T (cTnT), an integral component of the contraction machinery, shows decreased levels in Platr4-knockout EBs. Further, morphological abnormalities of EBs were observed with smaller size and a darker cavity in Platr4-knockout cells. Moreover, the relative expression levels of transcripts expressed in endoderm (Sox17, Foxa2) and mesoderm (Tbx5, Gata4) were markedly reduced in Platr4- depleted cells compared to control. In addition, deletion of Platr4 in mouse ESCs exhibited a significantly reduced expression of the cardiovascular gene network during targeted differentiation of cardiomyocytes. Therefore, our findings indicate that Platr4 is an important lncRNA regulator of lineage commitment during mammalian development.

F-2067

PERVASIVE DISCORDANCE BETWEEN MRNA AND PROTEIN EXPRESSION DURING EMBRYONIC STEM CELL DIFFERENTIATION

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During in vitro differentiation, pluripotent stem cells undergo extensive remodeling of their gene expression profiles. A large body of work has focused on transcriptional regulation, charting transcriptome changes during differentiation, most recently even at the single-cell level. Such studies assume implicitly that mRNA levels are a good proxy for protein expression and hence faithfully represent the gene expression profile. Mounting evidence suggests that this might not be a good assumption for mammalian systems, where mRNA and protein levels were found to correlate only moderately. Here we present integrated, genome-wide measurements of mRNA and protein levels during retinoic acid-driven differentiation of mouse embryonic stem cells. Most importantly, our study revealed pervasive discordance between mRNA and protein expression. Through a combination of systematic classification and kinetic modeling, we found a transient imbalance due to delayed protein synthesis or degradation as the source of most mRNA-protein discordance. The high temporal resolution of our combined RNA-seq and quantitative mass spectrometry measurements further allowed us to extract protein synthesis and degradation rates genome-wide. Additionally, we also identified candidates for dynamic regulation at the protein level. In-depth analysis of several gene sets revealed that cell type specific genes show a high concordance between mRNA and protein dynamics, while for ribosomal proteins the correlation is much lower. Our study thus supports a model in which mRNA fold changes set the level of newly produced proteins that have crucial, specific function in the differentiated cell types. Regulation on the level of protein turnover, on the other hand, is used to adapt the existing proteome. We finally show that our kinetic model can also predict protein abundances in purified, differentiated cell types. Together with our recent single-cell transcriptomics study of the same system, our work provides the most detailed characterization to date of mRNA and protein expression dynamics during in vitro differentiation.

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F-2069

DYNAMICS OF OCT4 SIGNALING IN HUMAN EMBRYONIC STEM CELLS

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Understanding the biology of human stem cells is critical for studies of development, tissue regeneration, and disease. Here, we used time-lapse fluorescence microscopy to visualize the dynamics of the core pluripotency factor OCT4 in human embryonic stem

cells. In order to carry out these studies, we developed a live-cell reporter system to visualize expression of human OCT4, a canonical marker of the pluripotent state. We used CRISPR-mediated genome editing to fuse a monomeric red fluorescent protein (mCherry) to the endogenous OCT4 protein in WA09 (H9) hESCs and isolated a clonal population of single-allele knock-in reporter cells. The OCT4-mCherry fusion protein showed accurate co-localization with endogenous OCT4 protein and was bound to the same promoter regions of OCT4 target genes. Cells bearing the OCT4-mCherry reporter were competent to differentiate into multiple differentiated cell types, and time-lapse imaging did not significantly affect their proliferation characteristics. By measuring OCT4-mCherry over multiple cell cycle generations, we identified a key regulatory event that alters a cell's propensity to either undergo self-renewal or differentiation. Based on these observations, we propose a model of stem cell differentiation in which the fate of an individual stem cell can be predicted from its OCT4 signaling pattern. We utilized a trophectoderm differentiation protocol to test this hypothesis and found that indeed cells were more likely to differentiate into trophectoderm based on their OCT4 expression dynamics. More broadly, our work provides a single-cell perspective on the dynamic balance between self-renewal and pluripotency.

F-2071

DEVELOPMENT OF A ROBUST DIFFERENTIATION METHOD TO INDUCE HUMAN IPSCS/ESCS INTO NEPHRON PROGENITOR CELLS USING TWO-DIMENSIONAL CULTURE

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Nephron is the basic structural and functional unit of the kidney, which maintains water balance and concentrations of soluble substances within the animal body. Based on the developmental findings of mouse embryos, the SIX2(+) precursor of the metanephric mesenchyme is derived from intermediate mesoderm through Brachyury(+)HOXD11(+) posterior primitive streak. Studies of kidney regeneration using stem cells have rapidly progressed in recent years. However, to achieve more improved organ regeneration and disease modeling, efficient methods to generate nephron progenitor cells (NPCs) that accurately reproduce developmental steps are required. We aim to establish an efficient protocol for differentiating hiPSCs into multipotent nephron progenitor cells that can form nephron-like structures using two-dimensional culture. We have established a robust differentiation method to induce human iPSCs/ESCs into SIX2(+) NPCs using two-dimensional culture. Furthermore, we use approaches to differentiate hiPSCs into posterior primitive streak fates by coarsely patterning HOX expression, thereby generating cultures specifying intermediate mesoderm regions. These SIX2(+)HOXD11(+) NPCs expressed other NPC marker genes and could differentiate into nephron constituent cells, including podocytes, proximal and distal tubular cells in vitro. Our differentiation protocol can induce human pluripotent stem cells into nephron progenitor cells with similar developmental potential to those in embryos, supplying systems for understanding the mechanisms of kidney development. Further, our patterning approach will enable detailed researches of HOX regulation in vitro, which could be used to conduct more informative studies of HOX patterning in kidney.

F-2073

EMERGENT BEHAVIOR IN NEURAL ROSETTES DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS)

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Neural rosettes are highly symmetric circular structures of polarized cells. In vitro, under the proper conditions, hiPSCs form rosettes that mimic the early stages of the embryonic spinal cord. Rosette formation is driven by fundamental molecular processes, but it is unknown how these generate the observed spatial organization. Recent studies discuss different types of emergent behaviors in cell populations, i.e. patterning in embryos, and the order/disorder transition in epithelial cells. These emergent phenomena are the result of stochastic cell behavior, and simple interactions. We hypothesize that rosettes are not the result of an explicit genetic code nor of deterministic cell commitment, but that rosettes are emergent and self-organized systems. By co-culturing cells with fluorescent nuclei and not-fluorescent nuclei, we have discovered that rosettes may be composed of cells derived from distinct progenitors. To study emergent behavior in rosette formation, we have designed a setup composed of a epifluorescence microscope and a stage-top CO2 incubator that are capable of acquiring time-lapse sequences over a period of 1-2 weeks. This set-up results in a 2 mm field width which allows us to capture the emergence of neural rosettes from the very beginning. In the image analysis, first the fluorescent

cell nuclei are detected from each image; then the tracking algorithm constructs the cell trajectories and lineage trees from the detected cell nuclei. Finally, we define an order parameter that quantifies the degree of organization and symmetry of the emerging rosettes and characterize the phase transition of the cell population. Preliminary results tell us that rosettes are emergent cells structures. We plan to construct complete lineage trees of rosettes' cells, and to mathematically model the mechanism that characterizes the fate decision making and the signaling of this system. The derived mathematical models will generate rosette formation predictions which we will compare with the observed expression of a fluorescent reporter under the control of the PAX6 regulatory sequences. These observations will help us mathematically model the correct development rosettes. In the long term our system might be useful to understand the basis of neurodevelopmental diseases deriving from neural tube defects.

F-2075

GENE EXPRESSION ANALYSIS DURING HUMAN STEM CELL CARDIOMYOGENESIS THROUGH POLYSOME PROFILING

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Differentiation of embryonic pluripotent stem cells (ESCs) is a highly coordinated process involving trigger, maintenance and coordination of gene expression patterns. Previous analyses in eukaryotes comparing mRNA and protein levels have indicated that there is no direct correlation between transcript levels and protein synthesis due to posttranscriptional regulation. Therefore, we aimed to analyze the mRNA fraction associated with translating ribosomes (polysomes) as a strategy to investigate posttranscriptional mechanisms involved in human ESCs (hESCs) under cardiomyocyte differentiation. NKX2-5eGFP/w hESCs were differentiated to cardiomyocytes and submitted to a time course analysis (D0, D1, D4, D9 and D15 days of differentiation). Pluripotency, mesodermal and cardiac markers were used to follow the cardiomyocyte differentiation by flow cytometry, immunofluorescence and RT-PCR. In addition, both the free and polysome mRNA subpopulation fractions were isolated using a sucrose density gradient and the polysome profile was recorded. cDNA libraries were prepared for high-throughput sequencing using the isolated mRNAs. RNA-seq analysis allowed the identification of gene regulatory networks and pathways involved in hESCs cardiomyocyte differentiation. Comparison between consecutive time points showed 10% (D1), 14.6%, (D4), 20% (D9) and 10.4% (D15) of differentially expressed genes and revealed that the larger change in expression happened during the cardiac progenitor shift (D9). We also compared free and polysome mRNAs in order to identify posttranscriptional regulated pathways. Genes considered as putative regulators are currently been investigated through overexpression studies. Our results will contribute to a better understanding of the key regulators and posttranscriptional mechanisms involved in human cardiac differentiation and development.

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F-2077

POTENTIAL OF HIPSCS DERIVED FROM DIFFERENT CELL TYPES TO DIFFERENTIATE INTO FUNCTIONAL ENDOTHELIAL CELLS

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Human induced pluripotent stem cells (hiPSCs) are currently considered as the cells which will be widely used in the regeneration of many tissues and organs. One possible utilization is their differentiation into the vascular endothelium. In our laboratory, we have derived hiPSCs from four different cell types. For derivation of hiPSCs we have used as a source cells: neonatal fibroblast, PBMC fraction, HUVECs and HSVECc. On these four hIPSCs lines we tested and evaluated two types of differentiation protocols into endothelial cells. First protocol "A" was aimed on direct differentiation into the lateral mesoderm with subsequent differentiation into the endothelium. In second protocol "B" we have incorporated step of controlled support of differentiation into primitive streak. In case of the first protocol, only up to 31% of cells had desired surface markers (SM) prior to separation. The second protocol yielded up to 48% of cells with desired SM. Quality of differentiated cells in following passages also varied greatly. There was a significant emergence of fibroblast-like cell (FLC) in cell culture by protocol "A". In such cases, if endothelial cells are not rescued via separation based on SM CD31/CD144 they are overgrown by FLC in matter of days. In passages following initial separation, the cells had 60-80 % of desired SM for the most part, they also needed to be re-separated to maintain these levels of SM. Even though FLC sometimes appeared in cell cultures by protocol "B", they grew at much slower pace and the rescue separation was as a result much less urgent. Levels of desired SM were also steadily rising even without separation up to 99%. These cells were positive for SM CD31, CD144, CD304 and CD34. Thus differentiated cells showed 100% efficiency on absorption of LDL and tube formation assay. Among the

individual hiPSCs lines, we have not found a fundamental difference in the yield between these two protocols.

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PLURIPOTENT STEM CELL: DISEASE MODELING

F-2079

INVESTIGATING NON-CELL AUTONOMOUS MECHANISMS OF NEURODEGENERATION IN SPINAL AND BULBAR MUSCULAR ATROPHY USING A HUMAN STEM CELL MODEL

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SBMA is a neuromuscular disease caused by a CAG repeat expansion (translated into glutamine, [Q]) in the androgen receptor (AR) gene, for which there remains no effective treatment, despite several clinical trials. The main pathological feature of SBMA is the progressive loss of motor neurons (MNs) that manifests as muscle atrophy of facial, bulbar (used for speech and swallowing), and proximal limb muscles. It is thought that the polyQ expansion results in aggregation of the AR protein and disruption of its normal function, and neuronal cells are particularly sensitive to such aggregates. However, the role of supportive cell types in neurodegeneration has been increasingly appreciated. Previous work from our lab established a mouse model of SBMA and showed that expression of mutant polyQ-AR in skeletal muscle is necessary for disease pathogenesis, as mice expressing polyQ-AR in all tissues – except skeletal muscle – did not exhibit hallmarks of SBMA, unlike littermate controls, thus supporting a central role for polyQ-AR expression in skeletal muscle in driving MN degeneration in SBMA. However, the causal molecular pathways and specific mechanisms remain unknown. Furthermore, it is unclear if the disease process and associated mechanisms can be recapitulated in a human cell context. To address these questions, we have established an induced pluripotent stem cell (iPSC)-based model in which both skeletal muscle and MNs are derived from SBMA patient or control cells. Differentiation of iPSCs into skeletal muscle using traditional protocols that aim to mimic the in vivo developmental process (such as those for MNs) has been challenging and inefficient. Thus, to circumvent this, we engineered iPSCs to express the myogenic master regulator MyoD and chromatin remodeling component Baf60c, resulting in a near homogeneous differentiation into skeletal muscle cells. iPSC-derived skeletal muscle can be assessed for SBMA-specific myopathic changes, co-cultured with MNs, and analyzed for transcriptome changes by RNA-Seq – with no contamination from non-myogenic cells. We predict that this new model of SBMA will be crucial to determine how skeletal muscle contributes to MN degeneration and for the future development of novel disease-modifying therapies for SBMA and perhaps other related MN disorders.

Funding Source: NSF Graduate Research Fellowship Program

F-2081

MODELING PAINFUL NA-CHANNELOPATHIES USING CRISPR/CAS9

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Pain is a critical adaptive sensation required for protection against danger and which facilitates healing after injury. However, when this system functions abnormally, either as a consequence of congenital or acquired abnormalities, pathological pain is generated. Mutations in ion channels expressed in sensory neurons have a major impact on pain sensibility. Among them, SCN9A which encodes Nav1.7 have been widely studied because it is the most abundant voltage-gated sodium channel in these "pain" sensory neurons. Gain-of-function mutations in Nav1.7 lead to inherited pain disorders including inherited erythromelalgia (IEM) and paroxysmal extreme pain disorder (PEPD), whereas loss of function mutations cause congenital insensitivity to pain (CIP). Most studies investigating the pathophysiology of Nav1.7 mutations do this by overexpressing human channels heterologously in non-neuronal cells or in rodent sensory neurons in vitro, which limits translational implications. We have successfully exploited CRISPR/Cas9 genome editing to introduce gain-of function (GOF) and loss-of-function (LOF) point mutations in the SCN9A gene to generate IEM, PEPD and CIP models in vitro (primary mouse and human induced sensory neurons) and in vivo (mutant mice) and have made multiple lines with these mutations. Nav1.7 GOF mutations in human iPSC induced sensory neurons dramatically increased spontaneous firing and burst firing rate in a temperature dependent manner as measured using multi-electrode arrays (MEA). Female mice carrying a Nav1.7 GOF mutation related to IEM in humans had difficulty nursing pups due to the pain and swelling of teats when

lactating. For Nav1.7 LOF mutation studies, we have performed MEA and whole-cell patch clamp recording and observed less spontaneous firing (MEA), less Nav1.7 currents and an increased threshold to generate action potentials. We also observed a high mortality in pups carrying Nav1.7 LOF mutations, similar to that observed in Nav1.7 knockout mice. These genetically engineered pain murine and human models not only enable us to mimic key features of the clinical presentation of the genetic pain disorder, but also provide a powerful tool to investigate pathophysiological mechanisms of the pain phenotype and to screen novel therapeutics for channelopathies.

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F-2083

GENERATION AND CHARACTERIZATION OF RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS CHONDROGENIC PELLETS USING SYNOVIOCYTE-DERIVED INDUCED PLURIPOTENT STEM CELLS

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Cartilage damage can lead to arthritic conditions in the knee joint. The cause behind the symptoms is the main difference between osteoarthritis (OA) and rheumatoid arthritis (RA). OA is usually caused by constant mechanical stress, trauma or natural aging. RA, on the other hand, is categorized as an autoimmune disease. Chondrocytes and fibroblast-like synoviocytes (FLSs) are the major cell types that reside in the synovial joint. FLSs are also infamous for its pathological role in RA. Therefore, it is thought to be closely related to the symptoms and the pathology of arthritis. In this study, human induced pluripotent stem cells (hiPSCs) were generated from FLSs isolated from the knee joint of RA and OA patients. Since the first demonstration of hiPSCs in 2007, it became a representative cell material for in vitro disease modeling. Using the disease-specific hiPSCs, the RA and OA chondrocytes was characterized. The generated hiPSCs were differentiated into cartilage-resembling chondrogenic pellets using pellet culture. Here, we compared the phenotype of RA and OA chondrocytes and chondrogenic pellets and suggest FLS-derived hiPSCs as a cell source for research on arthritic diseases.

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F-2085

PHOSPHOPROTEOMICS FROM HUMAN IPSC-DERIVED NEURONS IDENTIFIES A NOVEL LINK BETWEEN LRRK2, TAU, AND ALPHA-SYNUCLEIN

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Parkinson's disease (PD) is one of the most common neurondegenerative diseases, and novel therapies are urgently needed. LRRK2 G2019S is the most common genetic cause of PD, and increases kinase activity. Consistent with this LRRK2 inhibitors are being developed as possible therapeutics. To better understand the mechanisms of mutant LRRK2-induced PD, we generated induced pluripotent stem cells (iPSCs) from PD patients harboring LRRK2 G2019S as well as isogenic gene-corrected controls. Previously, we demonstrated that iPSC-derived midbrain dopaminergic neurons (mDANs) recapitulate key aspects of PD pathogenesis, including increased degeneration and elevated levels of α-synuclein. Inhibition of LRRK2 kinase activity with LRRK2 kinase inhibitors protected against PD pathogenesis, suggesting that LRRK2 actively phosphorylates an unidentified disease-relevant target. We used phosphoproteomics to identify putative targets of LRRK2 in human mDANs and found that LRRK2 kinase activity was required for the phosphorylation of multiple cytoskeleton-associated proteins, including TAU. This is interesting because GWA studies identified mutations in the MAPT locus as a risk factor for PD, and TAU pathology has been reported in some PD patients with mutant LRRK2. Consistent with the presence of cytoskeletal defects, we observed a severe organelle trafficking defect for mutant LRRK2 mDANs. Interestingly, LRRK2 kinase inhibition resulted in a complete phenotypic rescue. In addition, we knocked out LRRK2, which also rescued the observed trafficking defect, demonstrating that rescue was not an off-target effect. The microtubule-associated protein TAU governs axonal transport, and phosphorylation was decreased in a LRRK2-dependent manner, suggesting a potential link between trafficking defects and LRRK2. To test this, we used gene editing to abolish a specific TAU phospho-site that was LRRK2-dependent. mDANs with G2019S pTAU-mutant showed

partially ameliorated trafficking phenotype compared to G2019S alone. Interestingly, also the levels of α-synuclein were significantly decreased in pTAU mutant mDANs compared to G2019S. These results suggest a novel role of LRRK2 in the maintenance of axonal stability by regulating TAU phosphorylation and preventing the initiation of α-synuclein pathogenesis.

F-2087

CHARACTERIZATION OF NEURODEVELOPMENTAL ABNORMALITIES IN HUNTINGTON'S DISEASE USING INDUCED PLURIPOTENT STEM CELLS

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Huntington's disease (HD) is an inherited neurodegenerative disease and is characterized by atrophy of certain regions (mainly striatum and cortex) of the brain in a progressive manner. HD patients experience behavioural changes and uncontrolled movement which can be primarily attributed to the atrophy of striatal neurons. We have previously published models of the HD striatum using induced pluripotent stem cells (iPSCs) derived from HD patients. In this model, the HD iPSC-derived striatal cultures contained a high number of nestin expressing progenitor cells (neNPC). This "persistent" HD neNPC population has been found to be susceptible to stressors such as BDNF withdrawal. Similar upregulation of key neurodevelopmental signaling has been seen in various HD animal models. This population of aberrant neural progenitors is postulated to reflect a potential overall issue in maturation in HD. It was therefore hypothesized that the striatal maturation in HD is delayed and hence the resulting development abnormalities adversely affect the neuronal homeostasis hampering striatal neurons. This study was designed to further characterize this aberrant neural progenitor population and to characterize the mechanism of such delayed maturation.

F-2089

A COMPREHENSIVE DISEASE-SPECIFIC IPSC LIBRARY IN JAPAN

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Since induced pluripotent stem cells (iPSCs) were established by Prof. Yamanaka at 2007, the developing of new therapy, treatment and drug are desired in the incurable deceases. Recently, human disease-specific iPSCs and healthy donor iPSCs are developing rapidly on human iPSC bank, such as CIRM, HipSci, EBiSC, NYSCF, etc. However the variety of diseases on iPSCs are limited in those iPSC banks, since many of iPSC banks are focusing on some particular diseases. Therefore, we tried to make a comprehensive disease-specific iPSC library as a research resource in the world. We especially focused on intractable diseases listed by the Ministry of Health, Labour and Welfare in Japan, which are also able to analyze the disease-related phenotype in vitro. The patients of intractable diseases were recruited from the cooperative hospitals as private, university and national organization. We have established iPSCs from approximately 230 intractable disease, evaluated those iPSCs , and deposited on RIKEN BioResource Center, RIKEN BRC. A comprehensive disease-specific iPSC library will be useful for many researchers in the world.

Funding Source: he Program for Intractable Diseases Research utilizing disease-specific iPS cells of JST

F-2091

AN INDUCED PLURIPOTENT STEM CELL (IPSC) MODEL TO STUDY MECHANISMS OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) ASSOCIATED WITH PNPLA3 POLYMORPHISMS IN HUMAN HEPATOCYTES

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Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the adult and pediatric population. Genome-wide association studies (GWAS) have identified a polymorphism in the gene PNPLA3 that has a strong association with risk and severity of NAFLD. Using TAL effector nuclease (TALEN) technology, we generated isogenic lines from human induced pluripotent cells (iPSC) of known genetic background

with the variant and wildtype homozygous alleles of PNPLA3. These iPSCs grow well in culture and readily differentiate to hepatocyte-like cells (HLCs). To test the hypothesis that the variant PNPLA3 allele confer its risk due to aberrant lipid metabolism resulting in lipotoxicity in the early onset of NAFLD, we compared intracellular lipid accumulation by Nile Red triglyceride staining. We found that PNPLA3 variant HLCs accumulate lipid droplets at baseline and upon exposure to palmitic acid (PA), a toxic fatty acid that is prevalent in diets rich in saturated fats. PNPLA3 expression is reduced in variant HLCs, which is compensated by increased expression of other genes involved in lipolysis, such as PNPLA2 and PPARa. Variant HLCs also upregulate autophagy, which may be a consequence of lipid accumulation as autophagy has been proposed as a mechanism of lipid turnover in hepatic cells. To further explore the link between lipid accumulation and inflammation, we measured gene expression and secretion of cytokines that are relevant to the development of inflammation in NAFLD. We found that PNPLA3 variant HLCs have increased expression of IL1b at baseline, and increased secretion of IL1a and IL6 upon PA treatment. In summary, we have generated and validated the first known human model carrying polymorphisms specific to the PNPLA3 locus to study mechanisms of NAFLD. We have evidence that the variant allele of PNPLA3 contributes to aberrant lipid processing that may be the initiating even in NAFLD. We are currently testing compounds that ameliorate lipid accumulation and inflammatory phenotypes in variant HLCs with a focus on PPAR alpha agonists and autophagy inducers. Our work will open the door to a new range of experimentation in elucidating the mechanism underlying the association between PNPLA3 and NAFLD, predictive diagnostics and therapeutic discovery.

F-2093

GENERATION OF WERNER SYNDROME MODEL USING HUMAN PLURIPOTENT STEM CELLS

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Werner Syndrome (WS), also known as 'adult progeria', is a rare, autosomal recessive progeroid syndrome. WS patients exhibit various symptoms of accelerated aging such as growth retardation, short stature, premature graying of hair, alopecia, wrinkling, and skin atrophy. WS patients often suffer from severe ulcerations. A range of mutations in the WRN gene are known to cause WS. The human WRN gene is located on chromosome 8, and encodes WRNp protein which belongs to the RecQ helicase family. WRNp protein is thought to play an important role during DNA repair of double strand breaks, maintaining genome stability. In order to generate a cellular model for WS, one or both of the WRN alleles in human iPSC lines have been disrupted and a fluorescent protein gene was inserted using CRISPR-Cas9 technology so that the activity of the endogenous WRN promoter can be monitored and measured. The effect of single or double disruption of the WRN locus on cell survival and function is also being analyzed on various types of cells such as pluripotent cells, and cardiomyocytes and vascular endothelial cells differentiated from WRN-deficient iPSCs. This study will generate important clues to the pathogenesis of WS as well as the progression of normal aging.

F-2095

MODELLING DOPAMINE TRANSPORTER DEFICIENCY SYNDROME WITH PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

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Dopamine Transporter Deficiency Syndrome (DTDS) is an early onset progressive neurological disorder presenting with infantile parkinsonism-dystonia, due to loss-of-function mutations in SLC6A3, encoding the dopamine transporter (DAT). To investigate the cellular mechanisms underpinning DTDS, we generated induced Pluripotent Stem Cells (iPSCs) from two patients with different homozygous SLC6A3 missense mutations (p.L368Q and p.P395L) as well as from an age-matched control. All lines were successfully differentiated into midbrain precursors at high efficiency, with no significant differences between control and patient-derived cells. iPSC-derived midbrain precursors matured into electrically active dopaminergic (DA) neurons characterized by intrinsic spontaneous action potentials. Using High Performance Liquid Chromatography, an increased homovanillic acid (HVA):dopamine ratio was detected in the culture medium of patient–derived midbrain neurons when compared to control neurons. Notably, raised HVA levels are a key diagnostic disease feature reported in DTDS patient cerebrospinal fluid neurotransmitter analysis. When compared to control neurons, DA neurons from both DTDS patients had

decreased morphological complexity, and an overall reduction in neuronal population was evident in one patient. We also detected abnormal gene expression profiles for a number of genes involved in dopamine metabolism, including MAOA/B and COMT. In addition, our neuronal disease model of DTDS showed evidence of increased oxidative stress. Overall, our findings are suggestive of a neurodegenerative process in patient-derived DA, which may be a consequence of dopamine dyshomeostasis, secondary to DAT loss-of-function. In conclusion, we have demonstrated effective generation of mature dopaminergic neurons from two DTDS patients that has provided an ideal platform for studying cellular characteristics of the disease, as well as to develop novel therapeutic strategies.

Funding Source: Wellcome Trust

F-2097

MGH NEUROBANK: TOWARDS THE GENERATION OF A LARGE CELLULAR RESOURCE FOR STUDYING NEUROPSYCHIATRIC DISEASES

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A major limitation to understanding complex brain diseases is the inability to access brain cells from living patients. As a result, researchers often rely on indirect cellular models to study genetic or molecular mechanisms of disease, for example based upon blood cells or post-mortem tissue. Furthermore, these cell sources may be less amenable to manipulation such as introduction or rescue of genetic risk variants, assay development or drug screening. With the advent of iPSCs, the creation of genetically accurate cell lines from patients for the purpose of deriving neural cells to study neuropsychiatric disease has allowed for the in vitro modeling of brain disease. However, the complexity and polygenicity of most neuropsychiatric disorders require significantly greater numbers of patient lines across age, gender, ethnicity and clinical backgrounds to be confident in characterizing phenotypic differences between patient groups at the cellular level. To this end, we have generated an extensive bank of blood, fibroblasts, induced pluripotent stem cells (iPSCs) and neural progenitor cells (NPCs) lines from more than 400 skin biopsies including individuals diagnosed with neuropsychiatric diseases such as schizophrenia, bipolar disorder and major depression, as well as a large cohort of matched screened healthy control individuals without neuropsychiatric illness. These lines are linked to longitudinal electronic medical record data as well as a 2-hour neurocognitive testing battery and structured clinical assessment, including systematic assessment of treatment response history. By employing high-throughput phenotyping in large cohorts, this resource will facilitate association studies of cellular and clinical phenotypes and the first 150 fibroblast and iPSC lines have been deposited and will be made available to the research community through the NIH Rutgers repository.

F-2099

DEFINING RNA BINDING PROTEIN FUNCTION AND DYSFUNCTION IN HUMAN NEURONS

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Fragile X Mental Retardation Protein (FMRP) and its paralog Fragile X Mental Retardation Syndrome-Related Protein 1 (FXR1P) are RNA binding proteins which have been either directly or indirectly implicated in the pathogenesis of intellectual disability, autism and schizophrenia. These RNA binding proteins can repress translation of their target RNAs. However, their functions have not been well-defined in human brain cell types, which is crucial to fully understanding their contributions to disease and to the development of future therapeutics. Here, we utilize CRISPR-edited human pluripotent stem cells containing null mutations in FMR1 or FXR1 and their isogenic controls. Using an Ngn2-driven differentiation protocol, we compare human excitatory cortical neurons across multiple time-points to test a set of predictions about the impact on transcriptional, proteomic and electrophysiological measures following loss of each gene. These data will help to elucidate mechanisms of FMRP and FXR1P with additional human brain cell type and developmental specificity.

Funding Source: NIH 1 R21 MH109761-01A1

F-2101

MODELING HUNTINGTON'S DISEASE PATHOPHYSIOLOGY IN MUSCLE INDUCED HUMAN PLURIPOTENT STEM CELLS

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Huntington's disease (HD) is a fatal autosomal dominant genetic disorder caused by CAG trinucleotide expansions in the huntingtin protein producing abnormal aggregation and inclusion formation in tissues. Clinically HD is characterized by progressive cognitive and motor decline. Although classically viewed as a neurodegenerative disease, there is growing evidence suggesting that HD's pathology is at least in part associated with defects in muscle energy metabolism mediated by mitochondrial dysfunction (Chaturvedi, Adhihetty et al. 2009). Through a collaboration with Genea Biocells and the Bridges to Stem Cell Research Internship Program (BSCRIP), we present the development of a Huntington's disease human stem cell model for skeletal muscle. The unique disease modeling platform at Genea Biocells takes advantage of the world's largest bank of human embryonic stem cells (hESC) and a robust, high-yield myotube differentiation protocol (Caron, Kher et al. 2016). Using previously banked disease-affected HD hESCs with varying CAG repeat lengths, the scalable muscle differentiation protocol, and high-content imaging and oxidative stress screening platforms we report on a clinically relevant skeletal muscle model for HD.

Funding Source: California Institute for Regenerative Medicine

F-2103

DEVELOPMENT OF AN EBOLA VIRUS PATHOGENESIS MODEL USING IPSC-DERIVED HEPATOCYTES

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Ebola virus (EBOV) infection is known to cause severe human disease. Upon infection of the liver, measurable viral titers spike, and high viremia is associated with negative outcomes for patients. Therefore, we believe that the liver is a critical site for the successful life cycle of EBOV during human disease and potentially a good target for therapeutic interventions. Reston virus (RESTV) is an Ebola virus species that is replication competent but asymptomatic in humans. RESTV infection does not induce an interferon response in macrophages, but little is known about RESTV in the liver. Primary human liver samples are difficult to acquire, and animal models of EBOV infection either incompletely recapitulate disease or are costly. Our goal is to use iPSC-derived hepatocytes to create an easily reproducible system for modeling human EBOV infection and pathogenesis. We differentiated hepatocytes from human iPSCs and characterized them using flow cytometry, intracellular staining, qRT-PCR, and functional assays. Our hepatocytes expressed typical hepatic markers and CY3A4 was shown to be active. These cells were capable of binding LDL and contained LDL-rich vesicles. Once differentiated, our iPSC-derived hepatocytes with a recombinant vesicular stomatitis virus expressing the EBOV surface glycoprotein (VSV-Z76-GFP). Our cells were susceptible to infection. We also infected iPSCs and differentiated endoderm cells with VSV-Z76-GFP, and they were not susceptible to infection. During infection, our iPSC-derived hepatocytes expressed interferons and activated an antiviral response. Our iPSC-derived hepatocytes expressed surface markers that are involved in EBOV entry and differentially expressed these markers throughout the course of infection. We provide evidence that our iPSC-derived hepatocytes are capable of producing an antiviral response that can be used to model human EBOV infection. We can now use this model to better understand the mechanisms underlying the differential expression of interferons during EBOV and RESTV infection in the human condition. This system also allows us to investigate how the human liver supports EBOV replication and pathogenesis.

F-2105

INVESTIGATING ROLE OF APOLIPOPROTEIN E ISOFORMS IN ALZHEIMER'S DISEASE USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Late onset Alzheimer's disease (LOAD) is a progressive neurodegenerative disease and the clinical symptoms involve dementia and cognitive impairment. Recent genome-wide association studies (GWAS) identified more than 20 gene variants that are highly associated with LOAD and the $\varepsilon 4$ isoform of apolipoprotein E (ApoE4) is one of the major genetic risk factors. Human ApoE exists in three isoforms - ApoE2, ApoE3, and ApoE4 - with only two amino acid residue differences between the three isoforms. How the ApoE isoforms result in differential effects on AD pathogenesis remains

unclear. We applied the CRISPR-Cas9 genomic editing technique to generate ApoE3 and ApoE4 isogenic lines from both healthy individual- and LOAD patient-derived induced pluripotent stem cells (iPSCs). We then differentiated these isogenic iPSCs into different cell types, including neural progenitor cells, astrocytes, and neurons. Since ApoE has important roles in ligand delivery and receptor trafficking, we investigated whether ApoE isoforms have influence in the endocytic pathway. We found that the ApoE4 isoform results in endosomal abnormality in multiple cell types including astrocytes and neurons. We also performed transcriptome analysis in order to investigate the function of ApoE isoforms in transcriptional regulation and found changes in genes related to lipid homeostasis. Our data show that ApoE isogenic iPSCs are an excellent candidate to investigate the role of ApoE in Alzheimer's disease.

F-2107

USING HIPSC-DERIVED BETA CELLS TO ADVANCE DIABETES DRUG DISCOVERY

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By 2030, diabetes is predicted to be the seventh leading cause of death globally. Type I diabetes (T1D) is a chronic autoimmune variant of this disease characterized by pancreatic islet beta cell loss and dysfunction, which results in insufficient production of insulin and subsequent excess of blood glucose, leading to numerous complications. While advances in diabetes treatments, including the development of new classes of drugs, have made diabetes treatment more manageable, a gap in health outcomes between T1D patients and those without diabetes still remains. In addition to the development of new drugs, transplantation of pancreatic islets into patients, currently limited by donor availability, holds great promise for a diabetes cure. Stem cell-derived beta cells that faithfully recapitulate in vivo beta cell features have tremendous potential to advance diabetes treatments on both the drug discovery and regenerative medicine fronts. In vivo compounds known as incretins stimulate insulin production. Recent developments in therapeutics use incretin analogues, such as the GLP-1 analogue Exenatide, to induce insulin secretion. Finding new target receptors to treat diabetes requires in vitro models that secrete insulin in response to stimulation, like their in vivo counterparts. In particular, free-fatty acid receptors (FFARs) are under high scrutiny as likely candidates for new therapies. One strong therapeutic target of interest today is FFAR1 (GPR40). We have recently developed a hiPSC-derived beta cell line (ChiPSC12) displaying beta cell markers like insulin, C-peptide, MAFA, and NKX6.1. Here, we present data from another hiPS cell line, ChiPSC22, which carries the HLA type A*02:01 that is strongly associated with the susceptibility to develop T1D. We present further characterization of these cell lines, showing expression data of GLP-1R and FFARs together with analysis of insulin secretion upon stimulation with incretins and GPR agonist, to demonstrate their suitability for drug development. In addition, these newly developed beta cell lines can be used in a format suitable for High Throughput Screening (HTS), enabling a fast, reliable, and robust beta cell in vitro system for finding new diabetes therapies.

F-2109

IPSC-BASED MODELING OF CITRULLINEMIA, AN INHERITED UREA CYCLE DISORDER OF THE LIVER

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Induced pluripotent stem cells (iPSCs) hold great promise for cell-based therapies and modeling of human diseases. Here, we examined the use of human iPSCs for modeling Citrullinemia type I, an inherited urea cycle disorder of the liver that results from deficiency of the enzyme argininosuccinate synthase (ASS1). Patients suffering from Citrullinemia present in early infancy with life-threatening hyperammonemia that can be fatal or result in permanent neurologic damage. Despite the progress in pharmacologic management of the disease, long-term survival is poor for severe cases, and cell transplantation with functional hepatocytes is a promising therapeutic approach. In this study, dermal fibroblasts from citrullinemia patients harboring homozygous G390R mutations in the ASS1 gene were used to generate transgene-free disease-specific human iPSCs via episomal reprogramming. The resulting iPSCs were fully characterized with respect to pluripotency and then differentiated to hepatocytes using a novel 3-step differentiation protocol in chemically defined conditions. The resulting cells exhibited properties of mature hepatocytes, such as albumin secretion, cytochrome P450 metabolism, LDL uptake, glycogen storage as well as marker gene expression. Citrullinemia-specific hepatocytes generated increased levels of ammonia compared to control iPSC-derived hepatocytes. This disease-related phenotype could be reversed by the exogenous expression of the wild-type ASS1 gene in citrullinemia-hepatocytes. We therefore established a platform for hepatocyte generation and gene correction in citrullinemia-specific iPSCs. This model constitutes the foundation for pre-clinical studies in cell-based treatment of urea cycle disorders.

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F-2111

INSULIN RESPONSIVE GLUCOSE UPTAKE IN HPSC-DERIVED SKELETAL MUSCLE

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Skeletal muscle is the most abundant tissue in the human body. Accordingly, diseases that affect skeletal muscle cells can have a devastating effect on those afflicted. In order to develop new therapies for muscle-related diseases, we established a differentiation workflow for generating skeletal muscle from human pluripotent stem cells (hPSCs). This proprietary process involves transitions through myogenic progenitor and myoblast states followed by post mitotic fusion of myoblasts to multinucleated myotubes. Here we show that one of the key features of primary skeletal muscle, glucose uptake in response to insulin, is present in our hPSC-derived skeletal muscle. In addition, differentiated cells express high levels of both GLUT1 and GLUT4, important glucose transporters involved in the response. These findings support the ideal that hPSC-derived skeletal muscle recapitulates many of the characteristics of primary skeletal muscle and is therefore is an appropriate model for studying muscle-related diseases.

REPROGRAMMING

F-2115

NEURONAL DIFFERENTIATION AND IN VIVO FUNCTIONALITY OF A HUMAN NUCLEAR TRANSFER ES CELL LINE (NT4-HESC)

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Human embryonic stem cells generated by somatic cell nuclear transfer (SCNT-hESC) can provide immense opportunities for personalized medicine as they won't cause immune rejection problems. In this study, we investigated the neuronal differentiation and in vivo functionality of a nuclear transfer ES cell line (NT4-hESC), in parallel with human ESC and induced pluripotent stem cell (iPSC). First of all, we investigated whether NT4-hESC can spontaneously differentiate into mature neurons. Immunocytochemical analysis indicates that NT-4 can express various neuronal and synaptic markers, including Tuj1, MAP2, TH, GABA, SVP38, etc. Electrophysiological analysis based on whole-cell patch clamp recording further revealed that NT4-hESC-derived neurons express action potentials and sodium current at 12 weeks of differentiation. These neuronal and electrophysiological properties were essentially the same as those of ESC and iPSC-derived neurons. We next investigated the in vivo functionality of NT4-hESC-derived neurons following transplantation into a rodent model of ischemic stroke made by middle cerebral artery occlusion (MCAo). Behavioral analyses, based on rotarod, stepping, mNSS, stepping and apomorphine-induced rotation tests, clearly indicate that transplanted NT4-hESC-derived neurons can significantly improve the motor and sensory deficits in stroke-damaged animals. These behavioral improvements were comparable to the results of animals transplanted with either ESC or iPSC-derived neurons. Histological analysis further revealed that transplanted cells differentiated into MAP2-positive mature neurons, GABAergic neurons, DARPP32-positive medium spiny neurons, etc. at 12 weeks after transplantation. We also found that transplanted animals exhibited a significant increase of neurogenesis and angiogenesis. Taken together, these results strongly indicate that NT4-hESC can differentiate into mature and functional neurons efficiently, which, in turn, can significantly contribute to the behavioral recovery in stroke-damaged animals, providing the experimental evidence for the feasibility of SCNT-hESC in future therapeutic applications.

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F-2119

THE ROLE OF SOX2 IN INDUCED PLURIPOTENCY

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Sox2 is a master transcription factor in pluripotency, and it is one of the original Yamanaka reprogramming factors. However, the role that Sox2 plays in the process of induced pluripotency is currently not understood. Many pluripotency factors previously thought to be required for the induction of pluripotency have been demonstrated to be dispensable following improved

reprogramming protocols. However, this has not yet been investigated for Sox2. In addition, the molecular mechanism by which Sox2 contributes to induced pluripotency is unclear. To investigate these questions, we successfully generated Sox2-/- neural stem cells using CRISPR/Cas9 technology. These cells were used to test the requirement for Sox2 by rescuing with candidate transgenes, including a Sox2-2a-Cherry construct to allow monitoring of the expression levels of Sox2 in successfully reprogrammed iPSCs. This demonstrated that the iPSCs converge on a narrow range of Sox2 expression, despite varying the range of Sox2 expression in the initial reprogramming population. We generated embryonic stem cells with increased Sox2 expression levels, which was associated with a strengthened pluripotency transcription network, including a 1.9 fold increase in Nanog expression. The results of this work provide new insights into the role of Sox2 in induced pluripotency.

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F-2121

TRNASCRIPTOME ANALYSIS OF 3-D SPHEROID CULTURE OF HUMAN FIBROBLASTS REVEALS POTENTIAL OF THE METHOD IN INDUCING STEM CELL REPROGRAMMING

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Compared with 2-D cell culture, 3-D spheroid culture methodology is regarded as more physiologically similar to the native environment in the body. Numerous reports showed spheroid culture of mesenchymal stem cells (MSC) and cancer stem cells acquired enhanced differentiation capacity, sensitivity to chemicals, and elevated expression of differentiation- and stemness-associated genes. Despite this emerging trend, to date, there is no comprehensive study conducted on the effect of spheroid aggregate cell culture compared to traditional 2-D culture. To investigate into this topic, we used hydrogel-coated culture wells to force human skin fibroblasts forming into spheroid aggregates. Using whole genome RNA sequencing coupled with qPCR validation, we have detected more than 200 differentially expressed genes. Gene Ontology (GO) analysis revealed a majority of the genes are related to extracellular matrix composition and organization, and cell migration. Intriguingly, a number of early to mature stem cell markers and Mesenchymal-to-Epithelial Transition (MET) genes were significantly up-regulated while some of the Epithelial-to-Mesenchymal Transition (EMT) genes were down-regulated, resembling stem cell reprogramming. Similar phenomenon was observed across biological replications, cell types in both human and mouse samples, including somatic cells, MSC and embryonic stem cells. We also found that when used as coating of culture surface, the extracellular matrix of the spheroids could induce expression change of some genes in 2-D cultured fibroblasts in accordance to suspension culture. The result provides an insight in the biological significance of 3-D spheroid culture methodology over the traditional 2-D culture, and the potentials of using spheroid culture in stem cell reprogramming.

F-2123

ELECTROMAGNETIC FIELDS MEDIATE EFFICIENT CELL REPROGRAMMING INTO A PLURIPOTENT STATE

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Life on earth is constantly exposed to natural electromagnetic fields (EMF) and it is generally accepted that EMF may exert a variety of effects on biological systems. Particularly, extremely low frequency electromagnetic fields (EL-EMFs) affect biological processes such as cell development and differentiation, however, the fundamental mechanisms by which EMF influences these processes remain unclear. Here we show that that EMF exposure induces epigenetic changes that promote efficient somatic cell reprogramming to pluripotency. These epigenetic changes resulted from EMF-induced activation of the histone lysine methyltransferase Mll2. Remarkably, an EMF-free system that eliminates earth's naturally occurring magnetic field abrogates these epigenetic changes, resulting in a failure to undergo reprogramming. Therefore, our results reveal that EMF directly regulates dynamic epigenetic changes through Mll2, providing efficient tool for epigenetic reprogramming including the acquisition of pluripotency.

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F-2125

IDENTIFICATION OF NOVEL SMALL MOLECULES THAT PLAY IMPORTANT ROLES IN CELLULAR REPROGRAMMING PROCESS

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Pluripotent stem cells can be generated through the reprogramming of somatic cells, providing an infinite cell resource for use in regenerative medicine. The chemical approach to induced pluripotent stem cells (iPSCs) methodologies offers a promising strategy for clinical- grade iPSCs production. Here, we identified novel enhancers of iPSCs generation from selected FDA-approved compound libraries. One of the candidates, the peroxisome proliferator-activated receptor α (PPAR-α) agonist promotes the cellular reprogramming efficiency. We found that the PPAR-α agonist increases the expression of pluripotency-associated genes such as Oct4, Rex1 and Nr5A2 during the early stage of reprogramming process. Moreover the reprogramming-promoting function of PPAR-α occurs via the upregulation of Nanog which is essential to the induction and maintenance of pluripotency. Bioinformatic analysis showed that there are putative PPAR-responsive elements (PPREs) within the promoter region of Nanog gene. Taken together our findings may provide the new roles of the PPAR-α agonist as an iPSCs enhancer in reprogramming process as well as improve iPSCs technology.

F-2127

GENERATION OF INDUCED PLURIPOTENT STEM CELLS USING ELASTIN LIKE POLYPEPTIDE

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Induced pluripotent stem (iPS) cells have been generated from various somatic cells using different approaches; however, a major restriction of reprogramming methods is the use of potentially harmful genome-integrating DNAs. Here, without a viral vector, we generated iPS cells from fibroblasts using an elastin-like polypeptide (ELP)-based transfection method. Our findings support the possible use of ELPs for transfer of the reprogramming genes in to somatic cells for generation of iPS cells. After transfection, the iPS cells showed ES cell-like characteristics, including expression of endogenous pluripotency genes, differentiation into three germ layer lineages, and formation of teratomas. Our results demonstrate that ELP-based gene delivery may provide a safe method for use in generation of virus-free and exogenous DNA-free iPS cells, which will be crucial for future applications in cell replacement therapy.

F-2129

REPROGRAMMING TO CANCER INDUCED PLURIPOTENT STEM CELLS ELUCIDATES THE CONTRIBUTION OF GENETIC AND EPIGENETIC ALTERATIONS TO BREAST CARCINOGENESIS

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The induced pluripotent stem cells (iPSCs) technology has revolutionized disease modelling by enabling the generation of patient-specific pluripotent stem cells to enable the study of complex disorders such as cancer. Accumulative genetic and epigenetic alterations contribute to carcinogenesis and cancer progression. However, it is not clear their relative contribution to cell tumorigenicity. Somatic cell reprogramming induces global epigenetic reconfiguration of the chromatin with reversion of cancer cells to an embryonic stem cell-like state with potential loss of cell tumorigenicity. Therefore, reprogramming can be used to answer the question as to whether epigenetic alterations alone can be sufficient to induce carcinogenesis, independent of genetic defects. In this study, the triple negative breast cancer cell lines BT-549 and MDA-MB-231 were reprogrammed using non-integrating episomal vectors expressing OCT4, SOX2, L-MYC, KLF4 and LIN28. Partially reprogrammed cells (pre-iPSCs), expressing the pluripotency markers DPPA4 and FGF4, but not NANOG and REX1, were isolated. The expression of NANOG and REX1 were subsequently induced by treating pre-iPSCs with the demethylating agent 5-aza-2'-deoxycytidine. The reprogramming of cancer cell lines was greatly inefficient in comparison to the immortalized but not tumorigenic mammary cell line MCF-10A under the same conditions. Several iPSCs lines generated from MCF-10A were pluripotent and capable differentiating into the three germ layers. To study whether the refractoriness of cancer cells to reprogramming could be due to specific DNA mutations, we generated CRISPR-genetically modified MCF-10A cells harbouring the two most frequent clinically-relevant mutations found in triple negative breast cancer: PIK3CA_H1047R (+/-) and TP53 (-/-). The reprogramming of genetically modified MCF10A cell lines and the cancer cell line HCC1454, which harbours both mutations, will determine the contribution of PIK3CA and TP53 mutations to refractoriness to epigenetic reprogramming and will establish a model for the study of initiation and progression of triple negative breast cancer.

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F-2131

IDENTIFICATION OF SMALL MOLECULE REPLACERS OF GATA4 AND MEF2C IN CARDIAC REPROGRAMMING

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The direct conversion of fibroblasts into cardiomyocytes (iCMs) by forced expression of GMT (GATA4, MEF2C, TBX5) provides a novel approach for cardiomyocyte regeneration. However, genetic manipulation raised safety concerns in clinical application. It was shown that some small molecules were able to aid cell fate conversion and were sufficient to induce somatic reprogramming without genetic manipulation, which inspired new attempts to induce direct cardiac reprogramming with pure chemicals without genetic manipulation. In this study, we screened small molecules libraries of 3,000 small molecules with known targets or bioactivities, on mouse fibroblasts with alpha-MHC-mCherry reporter (alpha-MHC promoter driven mCherry expression), to identify small molecule replacers of transgenes that used in cardiac reprogramming. We found that 10 compounds (G1-10, including 6 inhibitors targeting a same kinase and 4 epigenetic modulators) were able to replace GATA4 to induce cardiac reprogramming in the presence of MEF2c and TBX5. Among these GATA4 replacers, G1 or G2 induced the highest efficiency by up to 60% of GATA4. Besides, these compounds increased reprogramming efficiency induced by GMT by up to 3-fold with improved kinetics. Moreover, we identified another small molecule (M1) that replaced MEF2C in inducing cardiac reprogramming in the presence of GATA4 and TBX5. ICMs induced with these small molecule replacers were further characterized to express typical cardiac maker genes, such as Troponin T, alpha-Actinin, Gap junction alpha-1 protein, Natriuretic Peptide A, and have similar gene expression patterns to that of primary cardiomyocytes. In conclusion, our findings leap a step forward to induce cardiac reprogramming and regeneration with pure chemicals. And the identification of small molecule replacers of GATA4 and MEF2C would help understand the underlying molecule mechanisms of cardiac reprogramming, as well as the functions of cardiac reprogramming genes.

TECHNOLOGIES FOR STEM CELL RESEARCH

F-2133

GAMMA IRRADIATION FOR TERMINAL STERILIZATION OF XENO-FREE CLINICAL GRADE HUMAN PLATELET LYSATE

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The use of fetal bovine serum (FBS) for clinical manufacturing of cell products poses risks for potential viral and prion transmission as well as adverse immunological reactions. Human platelet lysate (hPL) has recently emerged as a xenogeneic-free alternative to replace FBS in all steps of cell manufacturing. Compass Biomedical's PLUS™ hPL is produced under Good Manufacturing Practice (GMP) standards using expired platelet units from AABB-accredited blood banks. Although these platelet units are transfusion-grade and have undergone rigorous serology and infectious disease screening, contamination with currently undiscovered pathogens is possible. Therefore, a terminal sterilization process capable of removing or inactivating pathogens without a significant compromise in product performance is strongly desired. Among the terminal sterilization methods, gamma irradiation has several advantages, including high penetration depth, minimal temperature increase during processing, absence of residues post-treatment, and easy control of the applied dose. Since a growing number of blood-based products have adopted the use of gamma irradiation, we undertook this study to assess its impact on PLUS™. Briefly, 4 lots of GMP PLUS™ were gamma irradiated at four different doses ranging from 15-45 kGy. The samples were kept frozen on dry ice throughout shipping and irradiation. Post-irradiated products were compared with pre-irradiated controls in terms of appearance (color and presence of particulate), physiochemical profile (pH, osmolarity and total protein concentration), concentrations of important growth factors (VEGF, EGF, FGF-2 and PDGF-BB), and ability to promote growth of bone marrow-derived mesenchymal stromal cells (MSCs) in medium supplemented with 5% PLUS™. For all gamma doses tested, no changes in product appearance or physiochemical profile were observed. A dose-dependent reduction in growth factor concentration and MSC proliferation was observed, but some potency was maintained at 45 kGy. At 25 kGy, a commonly used dose for sterilizing medical devices, the drop in growth factor concentrations and MSC proliferation fold was less than 25%. This is a modest but

acceptable impact on the performance of GMP PLUS™ as a cell culture supplement, making it a safe product for use in cell therapies.

F-2135

IMPROVING THE PRODUCTION OF PLATELETS FROM HUMAN PLURIPOTENT STEM CELL DERIVED CONDITIONALLY IMMORTALIZED MEGAKARYOCYTE PRECURSOR CELLS

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In U.S., over 2 million platelet (plt) units are transfused every year to treat thrombocytopenia or plt dysfunction. The current standard of care, donor plt transfusion, suffers from several limitations, such as shortages, contamination, variable potency, and alloimmunization. Another alternative is large-scale manufacture of universal donor type product containing plts derived from megakaryocytes (MKs) in vitro. MKs can be generated from self-renewing human induced pluripotent stem cells (hiPSCs) or human embryonic stem cells (hESCs), potentially reducing or eliminating the need for human plt donors. Production of plts from hiPSCs is an inefficient process. Recently, hiPSC-derived immortalized MK progenitor cell lines (imMKCLs) have been produced by Eto and colleagues (CIRA) via doxycycline (DOX) dependent expression of cMYC, BMI1 and BCL-XL. Upon DOX removal imMKCLs mature & release functional plts. Our studies show that imMKCLs are heterogeneous with respect to a number of characteristics such as surface marker expression (CD41a, CD42b), maturation, and plt yield/cell. We developed a novel, high-throughput long-term imaging platform that allows us to observe individual imMKCLs as they mature and release plts. We discovered that rare individual imMKCLs produce plts at a rate of >300/cell in vitro. The burst-like plt release is not synchronized and the triggers are poorly understood. We also observed premature surface expression of CD62P (plt activation marker) on some maturing imMKCLs, highlighting need to generate imMKCL-subclones & better synchronize the release of plt. To address these issues, we developed an efficient single imMKCL subcloning method. Clones obtained postsorting and subcloning of CD41high-cells are more homogeneous and retain their sorted phenotype (CD41+CD42bhigh). When induced to differentiate, many of the subclones are enriched with imMKCLs that produced large amounts of plts. Our subcloning method has enabled use of CRISPR mediated genome editing of imMKCLs, making this platform ideal for testing role of candidate genes in MK/plt biology. We are using omics and chemical genetics approaches to understand the nature of observed heterogeneities and to identify new marker, cellular targets and triggers or probes that can be leveraged to improve the production of imMKCL plts.

Funding Source: Megakaryon

F-2137

A COMPREHENSIVE BIOPROCESS AND BIOECONOMICS MODEL FOR SCALE-UP AND SCALE-OUT OF MANUFACTURING OF MESENCHYMAL STEM CELL (MSCS) BASED THERAPIES

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Mesenchymal stem cell (MSC) based therapies are promising for a large spectrum of unmet medical needs. However, several factors are delaying the clinical adoption of these therapies. From a manufacturing perspective, challenges related to process scalability, labor intensive tasks, high GMP manufacturing costs and high product and process variability affect the quality and cost-effectiveness of the MSC-based products. Computational tools to simulate stem cell bioprocessing, incorporating biological and process variability and risk assessment at critical steps, are helpful to simulate rational strategies to handle uncertainty and reduce the manufacturing costs. This work presents a full bioprocess and bioeconomics model of stem cell manufacturing, including isolation, expansion, downstream and quality control steps. The model was developed through discrete event simulation with an open source programming language. The intrinsic equations and parameters that capture the cell biological features, according to the cell source and culture conditions, are embedded in the model. Biological, cost and process variability, as well as the risk of failure of the process at specific steps, are included through stochastic simulation. The scenarios of scaling up an allogeneic therapy and scaling out an autologous therapy are simulated for different demands and production strategies. Finally, the simulation results provide a statistical distribution of the total manufacturing costs per dose and strategies for containing these costs are evaluated.

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F-2139

GENE DELETION IN MICE AND MONKEY WITHOUT MOSAICISM BY CRISPR/CAS9 SYSTEM

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CRISPR/Cas9 system is an efficient gene editing method, but the majority of gene-edited animals showed mosaicism, with editing occurring only in a portion of cells. Here we show that single gene or multiple genes could be completely deleted in 100% of mouse embryos by zygotic injection of Cas9 mRNA and multiple adjacent single-guide RNAs that target only a single key exon of each gene. Phenotypic analysis of F0 mice following targeted deletion of eight genes of the Y chromosome individually demonstrated the robustness of this approach in generation of gene-knockout mice. Importantly, this approach produces complete gene deletion efficiently (100% on Arntl and 91% on Prrt2) in monkey embryos. Finally, we generated a monkey model for human paroxysmal kinesigenic dyskinesia by Prrt2 knockout in one step without mosaicism, demonstrating the usefulness of this approach in rapidly establishing gene-edited monkey models.

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F-2141

IDENTIFICATION OF A NOVEL HUMAN-SPECIFIC DEVELOPMENTAL REGULATOR

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Insulin is a small protein (51 aa) encoded by a small gene (450 bp) with a potent role in glucose control. Inspired by insulin, we developed an unbiased and novel strategy to identify functional small coding transcripts. Current molecular methods to identify new small genes encoding proteins are lacking. Indeed, most expression profiling isolation techniques select for genes larger than 200 bp or for microRNAs. Using "in solution" size fractionation of RNA, we robustly isolated small transcripts from diverse cell sources. After immuno-precipitation of small-capped poly-A transcripts, we enzymatically camouflaged them as miRNA to intersect with commercially available RNAseq technologies. Next, we applied our protocol to identify novel small transcripts that are early effectors of the TGF-β and WNT pathways and that are capable of inducing endoderm gene expression in Human Embryonic Stem (HUES) cells. We found a novel small transcript, Inducer of Definitive Endoderm 6 (IDE6), which is upregulated upon endoderm induction. IDE6's expression kinetics match that of known inducers of the endoderm lineage. It synergizes with Activin A to increase definitive endoderm induction in a WNT-dependent manner. The transcriptional activation of IDE6 by the TGF-β and WNT pathways was conserved in HEK293T cells. Using this cell line, we were able to show that V5-tagged IDE6 was translated into protein. Mass spectrometry analysis determined that IDE6 interacts with components of the RNA-induced silencing complex and with members of the SWI/SNF nucleosome remodeling complex. Surprisingly, we found a small conserved domain in IDE6 that we used to identify a novel family of proteins. In these proteins, the conserved domain has unprecedented positional bias towards the C-terminus. The novel protein family is enriched for Zinc finger domains, suggesting that they act in the nucleus. Phylogenetic analysis revealed that IDE6 and sequence homologues are exclusive to primates, highlighting the importance of using HUES cells to find novel human specific biology. Together, we demonstrate that this new methodology identifies novel and functionally significant small genes. This approach can be broadly applied to other systems for small protein discovery.

F-2143

OSTEOGENIC DIFFERENTIATION OF BONE-MARROW DERIVED MESENCHYMAL STEM CELLS IN DIFFERENT RELEASE SYSTEMS

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Bone morphogenetic protein-2 (BMP-2) is a well-known osteogenic differentiation factor that stimulates stem cell signaling pathways by activating transmembrane type I and type II receptors. However, BMPs have a very short half-life and lose their bioactivity quickly. Thus, a BMP delivery system is required to take full advantage of the induction stage for osteogenic differentiation. Several types of systems have been designed and evaluated but these methods were focused on carriers and sustainability. In fact, no studies have evaluated the effect of delivery mode in cell cultures during osteogenic differentiation. Therefore, the purpose of this study was to investigate the effect of different delivery approaches using bone marrow-derived mesenchymal stem cells (BM-MSCs). To that end, a bottom-up and top-down release system were fabricated for BMP-2 delivery. In this study, we selected RGD-conjugated alginate hydrogel for BMP-2 delivery because alginate is able to release BMP-2 in a sustained manner while also being biocompatible. After 7 days of culture, the bottom-up release system significantly stimulated alkaline phosphate activity of MSCs. These results may provide useful tools for expanding the potential applications of stem cell therapy.

Funding Source: This research was supported by grants from the Korea Health Industry Development Institute grants (HI14C3365) and the National Research Foundation funded by the Korean Government, MSIP (NRF-2016M3A9B4919616).

F-2145

ADULT BOVINE-DERIVED LEUKOCYTE- AND PLATELET-RICH PLASMA (L-PRP) IS SAFE, LESS ETHICAL, ECONOMICAL AND POWERFUL ALTERNATIVE TO FETAL BOVINE SERUM FOR THE CULTURE OF MESENCHYMAL STEM CELLS

Yamahara, Kenichi[1], Sudo, Toshita[2], Hamada, Akiko[3], Kuroda, Shoko[3], Yoshihara, Kyoko[3], Yoshihara, Satoshi[3], Okada, Masaya[3], Soma, Toshihiro[3], Ohnishi, Shunsuke[4] and Fujimori, Yoshihiro[3]
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Fetal bovine serum (FBS) is a common component of culture media and usually used for cellular research, as well as recent cell-based medical products. However, due to the high risk of contaminations and the variation from batch to batch, FBS might influence the outcome of research or cellular manufacturing. FBS also contains moral concerns because it harvested from bovine fetuses taken from pregnant cows. In addition, FBS is most expensive part of cell culture. To overcome these problems, we developed a new serum, adult bovine-derived leukocyte- and platelet-rich plasma (L-PRP). Using apheresis medical devices with closed disposable kits, sterile bovine L-PRP is collected from healthy bovine receiving a regular veterinary check. After stimulation and removal of coagulated fibrin by centrifugation, bovine L-PRP is collected in a completely closed system. To meet the scope of directives that apply to produce medicinal products from the European Agency for the Evaluation of Medicinal Products (EMEA/CVMP/743/00) and the United States Department of Agriculture (9CFR§113.420), bovine L-PRP is finally gamma-irradiated at a dose of 30 kGy. Similar to blood donation, bovine L-PRP can repeatedly obtain from one adult bovine without sacrifice, indicating less moral problem and lot-to-lot variation. In addition, bovine L-PRP costs much less than FBS. To test whether bovine L-PRP might be useful for mesenchymal stem cells (MSCs), a cell culture experiment was performed. 3 to 5 days after bovine PRP treatment, the proliferation of human bone marrow- and amnion-derived MSCs was significantly increased ($p < 0.05$) compared to FBS. Our results confirm that safe, less ethial and economial bovine L-PRP profoundly enhances MSC proliferation.

F-2147

GINICLUST: DETECTING RARE CELL TYPES FROM SINGLE-CELL GENE EXPRESSION DATA WITH GINI INDEX

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High-throughput single-cell technologies have great potential to discover new cell types; however, it remains challenging to detect rare cell types that are distinct from a large population. We present a novel computational method, called GiniClust, to overcome this challenge. Validation against a benchmark dataset indicates that GiniClust achieves high sensitivity and specificity. Application of GiniClust to public single-cell RNA-seq datasets uncovers previously unrecognized rare cell types, including Zscan4-expressing cells within mouse embryonic stem cells and hemoglobin-expressing cells in the mouse cortex and hippocampus. GiniClust also correctly detects a small number of normal cells that are mixed in a cancer cell population.

F-2149

ARRAYED MUTANT HAPLOID STEM CELL LIBRARIES FACILITATE POSITIVE AND NEGATIVE GENETIC SCREENS

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Forward genetic screens using mammalian embryonic stem (ES) cells have identified genes required for many cellular processes. However, loss-of-function screens are harder to conduct in diploid cells because in most cases, both alleles of a gene must be mutated to show a phenotype. Recently, mammalian haploid ES cell lines were successfully established in mouse, rat, monkey and human, which were also applied to generate the large pools of random mutations and to conduct a few small- or large-scale genetic screens. However, these screens were performed in mixed pools of mutant cells; therefore, the null mutants of interest had to be selected positively, which limited their application. Here, we developed a method for rapidly generating arrayed haploid mutant libraries using haploid murine ES cells and piggyBac (PB) transposon-based dual directional insertional mutagens, in which the proportion of homozygous mutant clones can reach 85% and most clones contain a single-copy transposon insertion. These key features allow the arrayed mutant libraries to be applicable for high-throughput phenotypic screening. Firstly, we utilized the arrayed library to screen mutant clones which showed resistant to differentiation under differentiation induced culture conditions. After screened 2,208 individual mutant clones, we got 69 candidate encoding genes. Among them, several known critical "exit-from-pluripotency" genes were included. Secondly, we conducted a negative screen of thousands individual ES cell clones to discover some possible new factors conferring sensitivity to DNA double-strand break (DSB) inducing drug, Doxorubicin. The screen identified dozens of DSB and DNA repair related genes, including a few known factors and several new candidates. Finally, by using the transposase-mediated reversion analysis and rigorous CRISPR/Cas9-based loss-of-function assays in diploid ES cells, we validated some of these candidates from the two screens mentioned above, and the high confirmation frequency demonstrated the availability and high efficiency of genetic screens on the arrayed mutation libraries.

Funding Source: This work was supported by grants from the National Key Research & Development Program of China (2016YFA0100103 to Y.H)

F-2151

IMMUNOCHARACTERIZATION OF CANINE AND FELINE ADIPOSE-DERIVED STEM CELLS BY QUANTITATIVE IMAGE FLOW CYTOMETRY

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Adipose-derived mesenchymal stem cells (adMSCs) are a potential cell source for autologous cell therapy due to their ability to self-renew, differentiate, modulate oxidative stress, secrete various cytokines and growth factors. These traits give adMSCs immunomodulatory, angiogenic, anti-inflammatory and anti-apoptotic qualities. The 2013 ISCT meeting defined human MSC cells as aplastic-adherent cells expressing specific surface markers including: CD105, CD90, CD73 or CD44 and CD29. Similar definitions have been published but there is no current consensus for animal stem cells. Thus the aim of this study was to characterize canine and feline adMSCs by the immunophenotypic properties and intra-cytoplasmic proteins responsible for non-differentiation. adMSCs were derived from subcutaneous fat tissue after a 30 min digestion in a solution containing 1 mg/ml collagenase I and 0.1 mg/ml BSA Fr V before filtering through a 100 µm and 40 µm cell strainer. Collagenase I inactivated by with a double volume of PBS. The cells were centrifuged for 10min at 300 x g and re-suspended in D-MEM low glucose 10% FBS and antibiotics, plated at a density of 2.5 × 10E4 cells/cm2 and cultured for 8 days in 5% CO2 at 37.5C. After 24hrs, non-adherent

cells were removed and adMSCs were cultured to 90% confluence before detaching with trypsin. Passage 1 cell samples from each animal were evaluated for immunophenotypic characterization using surface markers CD29, CD44, CD90 as well as cytoplasmic protein markers SOX2 and OCT3/4 by quantitative imaging multicolor flow cytometry (Amnis). At passage 1 osteogenic differentiation was induced. After 30 days, Alizarin O Red was used to detect extracellular calcium deposition. Following multiplex quantitative image flow cytometry analysis, isolated canine and feline adMSCs were found to be 89.33±1.61 and 94.99±0.36% triple positive for CD29, CD44 and CD90, 97.34±0.55% and 95.29±1.21% for OCT3/4 as well as 97.08±0.89% and 98.34±0.58% for SOX2, respectively. All of the cultures were positive for Alizarin O Red confirming the efficiency of our purification process reflected by the staining results. These results demonstrate the presence of the ISCT main specific markers in adMSC and can be applied in establishing a panel of markers for stem cell immunocharacterization for veterinary application.

F-2153

CARDIAC REGENERATION BY STATIN NANOPARTICLE-LOADED ADIPOSE-DERIVED STEM CELL TRANSPLANTATION IN MYOCARDIAL INFARCTION

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Clinical trials of autologous adipose-derived stem cell (AdSC) therapy for ischemic heart diseases (IHD) are now on-going. We have investigated the hypothesis that combination of AdSCs and statin, an agent with pleiotropic effects, could augment the therapeutic effect on myocardial infarction (MI). Human AdSC functions with different doses of simvastatin-conjugated nanoparticle (STNP) uptake were evaluated by in vitro assays. STNP promoted the migration activity without changing the proliferation activity, and also up-regulated growth factors. Next, MI was induced by LAD ligation in nude mice, and the mice were assigned in the following groups 3 days after MI: 1) PBS (control), 2) NP-AdSCs (50000 cells), 3) STNP, and 4) STNP-AdSCs (50000 cells). Cardiac functional recovery assessed by echocardiography was improved at 4 weeks after surgery in STNP-AdSC group. Masson's trichrome-stained sections revealed that LV fibrosis length was reduced, and the number of TUNEL-positive cardiomyocytes was less in STNP-AdSC group. Surprisingly, a number of de novo endogenous Nkx-2.5 positive immature cardiomyocytes as well as massive vascular formation were observed in outer layer of infarcted myocardium despite of a few recruited/retained transfused STNP-AdSCs 4 weeks after MI in STNP-AdSC group. Finally, massive myocardial regeneration was observed 8 weeks after MI. Intravenously injected small number of statin nanoparticle-loaded hAdSCs exhibited a potent therapeutic effect inducing endogenous cardiac tissue regeneration.

F-2155

GOOD CELL CULTURE CONDITIONS FOR HUMAN PLURIPOTENT STEM CELLS

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Pluripotent stem cells have been thought to be useful sources for regenerative medicine. Although embryonic stem (ES) cells have abilities to differentiate into several kinds of somatic cells and grow infinitely in vitro, there are several problems with using ES cells for clinical application. To overcome these problems, induced pluripotent stem cells (iPSCs) were generated from somatic cells. iPSCs have raised hopes for a new era of regenerative medicine because they can avoid the ethical problems and innate immune rejection. Human iPSCs are typically generated and maintained on feeder cells. Mouse feeder cells are conventionally used for hiPSC culture. These cells are prepared with FBS-containing medium. For clinical use, feeder-free (Ff) and xeno-free (Xf) culture conditions seem to be better than the conventional ones. We succeeded to develop the Ff-culture conditions by using recombinant laminin proteins and Xf-medium for hiPSC establishment and long-term culture. We could generate human iPS clones from fibroblasts and some kinds of blood cells. Under the novel feeder-free culture conditions, hiPSCs were stably maintained for 20-30 passages, and markers of pluripotency, such as Oct3/4 and TRA-1-60, were still detected at normal expression levels. These cells also have the differentiation ability to three germ layers and maintain the normal karyotype. We concluded that our novel culture method is sufficient and efficient for hiPSC culture. We are now trying to improve the feeder-free culture system for maintaining human pluripotent stem cells more stable condition.

Funding Source: This research was supported in part by a grant from Core Center for iPS Cell Research, Research Center Network for Realization of Regenerative Medicine from Japan Agency for Medical Research and Development, AMED and AJINOMOTO CO. INC.

F-2157

COMPARISON OF 3 DIFFERENT METHODS FOR DETECTION OF GENETIC STABILITY ON HUMAN PLURIPOTENT STEM CELLS

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For large scale banking facilities such as the UK Stem Cell Bank (UKSCB) and the European Bank for Induced Pluripotent Stem Cells (EBiSC) genetic stability has become an increasing important test for both safety and for information. It is essential to know if the banking process has altered the cells in any way that could affect their future performance and safety. With the growing number of disease specific, gene edited, and clinical grade stem cells and cell products being used in research and clinical trials, the unknown impact of a karyotype change remains a significant safety concern. The current gold standard for testing is full Giemsa-banded karyotyping of metaphase chromosomes. Here we report an investigation of the use of 3 different techniques for the evaluation of genome status; Bacs on beads (BOBs), Giemsa banding (G-banding) and a single nucleotide polymorphism (SNP) array on 12 different human pluripotent stem cell lines from the same bank of cells. Each of these methods differs in sensitivity, resolution and the ability to detect certain abnormalities. They also differ in cost and turnaround time. Of the 12 lines, only 3 gave exactly the same result when analysed by all 3 methods. BOB results indicate that this was the method of lowest sensitivity in our hands and did not detect changes seen using the other two methods. SNP array and G-banding, in general gave consistent results, but there were some lines where the results revealed discrepancies which could not be readily explained. This is the subject of ongoing investigation. The interpretation of inconsistent results from different techniques represents significant challenge for the acceptability of stem cell base therapies and emphasises the importance of a multifaceted approach to ensure confidence in genetic analysis.

Funding Source: INNOVATIVE MEDICINES INITIATIVE, EFPIA, Medical Research Council (UK), BBRSC

F-2159

IMPROVED TRANSGENE TARGETING IN HUMAN PSCS MEDIATED VIA CAS9 PROTEIN TRANSFECTION

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The advent of nuclease-assisted gene editing techniques, such as the CRISPR-Cas9 system, has markedly improved the efficiency and speed that genetic modification can be performed in human pluripotent stem cells (hPSCs). However procedures to precisely target transgenes, such as fluorescent proteins, to specific genomic loci remains inefficient, labour-intensive and time-consuming. Part of the issues relate to the cytotoxicity caused by the delivery of multiple plasmids encoding Cas9, the guide RNA (gRNA) and also the targeting construct containing the transgene(s) to insert into the hPSCs. Using a reporter hPSC line, we have developed an optimized lipofection-based procedure for the delivery of Cas9-gRNA ribonucleoprotein complexes (Cas9 RNPs) along with donor DNA encoding a fluorescent protein. Without including a selection step, ~5% of the cells have the fluorescent protein precisely inserted. Moreover, because of the low cytotoxicity these transfections can be readily performed with only 30,000 input cells in a 48-well format. If an enrichment step is included to target transcriptionally silent loci, up to 14% of the resulting cells have the transgenes inserted via homologous recombination in multiple hPSC lines. Combined with our clonal isolation procedure, this workflow enables the generation of clonal lineage-specific hPSC reporter lines in less than two months. Such improvements have the potential to support more complex genetic modifications to be performed in hPSCs at higher throughput.

Funding Source: Funding for this study was provided by an ERC Starter's Grant (STEMCARDIORISK; 638030) and a ZonMW VIDI Grant (Illuminate; 016.156.303).

F-2161

THE UTILITY OF MULTI COMPONENTS ANALYSIS OF CULTURE SUPERNATANT USING LC-MS/MS FOR MEDIA DEVELOPMENT AND IN-PROCESS MONITORING OF CELL CULTURE

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Cell culture media are one of the critical elements for the development and commercialization of regenerative medicines. Also enhanced control of the culture process is becoming important to reduce variability and improve consistency of manufacture. Multi-components analysis of culture supernatant can provide valuable information for this purpose, because it can identify which components the cells utilize for their growth and suggests which metabolic pathways are active. Current technologies for process monitoring with a focus on metabolism are usually limited to glucose/lactate/glutamine/ammonia but do not provide sufficient information for quality monitoring in more complex culture systems. We have developed a simultaneous analysis method for measuring the abundance of 95 compounds found in basal media and secreted metabolites using liquid chromatography-tandem mass spectrometry (LC-MS/MS). In this study, we used the CGT-RCIB10 iPS cell line (peripheral blood-derived) established by the Cell and Gene Therapy Catapult. Cells were maintained in Essential-8 medium on vitronectin-N coated plates for four days. As a control, medium only was incubated under the same conditions. The medium was exchanged every 24 hours and the spent medium was analyzed by LC-MS/MS. Cell confluency was also estimated by measuring total colony area at the same time. Good correlation (R2 > 0.9) was observed between cell confluency and consumption/accumulation of 13 compounds. These compounds and related metabolic pathways were assumed to be important for their growth. Our LC-MS/MS analysis methodology also showed hypoxanthine, which is one of the components of Essential-8, was depleted after 2 days in culture, though medium was exchanged daily. This results suggested the amount of hypoxanthine may impact cell growth particularly at higher confluency. The lack of significant differences between samples and controls of a number of amino acids and vitamins suggested that the cells did not consume these components for their growth. Thus, multi-components analysis of culture supernatant is useful for the further improvement of culture media. Moreover, our LC/MS analysis methodology provides valuable information for discovering key markers for in-process monitoring for cell culture.

F-2163

EVALUATION OF SMALL TO LARGE SCALE BIOREACTOR SERIES FOR IPS CELL STIRRED SUSPENSION CULTURE

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Induced pluripotent stem (iPS) cells are promising cell sources for regenerative medicine. The stirred suspension culture using a bioreactor system is an efficient method for the large scale expansion of human iPS cells. We designed a bioreactor series covering 100 folds the volume of culture from 5mL to 500mL. A small-scale bioreactor is suitable for screening assays for culture conditions, and a large-scale bioreactor is suitable for production scale verification. An important requirement for these applications is that correlated culture results are obtained on all scales. We evaluated bioreactor series using iPS cell proliferation, shape of aggregates, maintenance of undifferentiated markers as indicators. Four types (5mL, 30mL, 100mL, 500mL) bioreactors (ABLE Corp., Japan) were used for evaluation of iPS cell culture. These bioreactors were equipped with impeller of the same design that allowed for uniform stirring while preventing turbulence. These bioreactors were similarly equipped with stirring blades of the same design that allowed for uniform stirring while preventing turbulence. The impeller was a triangular paddle blade design and was suitable for floating aggregates. 5 mL and 30 mL vessels were simple bioreactors without sensors and were used in the CO2 incubator. 100 mL and 500 mL reactors can be equipped with sensors for temperature, pH and DO, and culture conditions are controlled by the device. The inoculated single cells aggregate under optimal culture conditions without microcarrier. The aggregates that grew after the 4-day culture period were collected. The size of aggregates was measured under microscope using software. The aggregates were dissociated into single cells using enzymes and the number of viable cells were measured. The undifferentiated property of the expanded iPS cells was evaluated with the flow cytometric analysis. As results, 5mL to 500mL scale culture using enabled to create a lot of aggregates 200 to 300 micrometer diameters in the cultivation for 4 days. Furthermore, the same cell proliferation was observed in these bioreactors

and the number of cells increased 5 to 10 folds for 4 days. We will discuss whether these results are suitable for screening to production scales verification purposes.

Funding Source: This research is supported by Projects for Technological Development in Research Center Network for Realization of Regenerative Medicine from Japan Agency for Medical Research and Development, AMED.

F-2165

IDENTIFICATION OF PREDICTIVE MARKERS FOR DIFFERENTIATION OF HUMAN IPS CELLS INTO ECTOMESENCHYMAL CELLS

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Induced pluripotent stem cells (iPSCs) are expected to contribute greatly to regenerative medicine and disease treatment. Several issues however remain to be resolved, one of which is their heterogeneity, in particular of differentiation potential. In our previous studies for chondrogenic differentiation through the neural crest cell (NCC) lineage, we used CD271high+ as a marker for ectomesenchymal cells, which further differentiated into chondrogenic precursors. Induction of CD271high+ population is therefore a crucial step for chondrogenic differentiation. The induction efficacy, however, varied considerably among clones, and it is currently impossible to predict which iPSCs are suitable for the induction of CD271high+ cells. Here we attempted to identify the biomarkers indicating such differentiation property of iPSCs by transcriptome analyses. We first induced CD271high+ cells from ten human iPSC clones and classified them into good or poor clones, judged according to their efficacy being above or below 20%, respectively. Gene expression profiles of these clones were obtained at iPSC stage. Approximately 1600 gene expressions were significantly different ($p < 0.05$) between good and poor clones. Furthermore, we focused on the 61 genes whose expression varied by at least 1.5-fold between good and poor clones. The discrimination model was constructed based on the data for these genes, and the significance was confirmed by the permutation test. Using this model, the mean induction score for good clones was 0.97, whereas those for poor clones was 0.04, indicating that we have established a candidate scoring system to evaluate the differentiation property of iPSCs toward ectomesenchymal cells. In the future, we will apply this model to the other iPSC clones to investigate whether we can prospectively predict the differentiation property of each iPSC and furthermore to identify key molecules involved in differentiation toward the ectomesenchymal cells.

F-2167

VALIDATING ANTIBODY SPECIFICITY WITH KNOCKOUT CELL LINES

Prater, Michael, Jakielaszek, Aneta, Koch, Sofia, Lochead, Julia, Bruce, David and Solache, Alejandra
Abcam, Cambridge, UK

Defining what an antibody binds to is crucial for generating reliable, reproducible results. Antibody validation techniques employed by antibody vendors typically include peptide ELISAs, reactivity against recombinant proteins, use of positive and negative control samples, and interference RNAi (RNAi). While these each have numerous benefits, they remain incapable of confirming antibody specific beyond all doubt. In response to this, we at Abcam are using human knockout (KO) models to interrogate the specificity of our antibodies on a large scale. This has been made possible with the extensive library of KO cell lines that were generated using CRISPR/Cas9 technology. By comparing antibody reactivity in a KO versus a wildtype sample, we can unequivocally show what our antibodies bind to within a cell. We confirm these results in either denatured (western blot) or native (immunocytochemistry) conditions. It is our hope that by using KO validation techniques we can help to raise industry standards and contribute to the generation of reproducible research.

F-2169

DEVELOPMENT OF A XENO-, SERUM-FREE EXPANSION MEDIUM FOR EX VIVO EXPANSION AND MAINTENANCE OF HUMAN HEMATOPOIETIC STEM CELLS

Lopes, Vanda Sofia[1], Kinjo, Jessie[2] and Ni, Jessie[2]
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Hematopoietic stem cells (HSCs) are the multipotent progenitors of the hematopoietic lineages and their use in regenerative medicine and gene therapy is a rapidly growing area. While much progress has been made in understanding their biology, the ex vivo expansion of sufficient HSCs with consistent quality for desired clinical application remains to be a challenge. A high performing HSC expansion medium closely meeting the clinical quality requirements becomes critical for achieving HSC's full clinical potentials. In this study we present a xeno- and serum-free media designed for expansion of HSCs. CD34+ cells were isolated from cord blood and expanded in various media prototypes supplemented with SCF, TPO, IL-3, IL-6 and Flt-3. The performance of

media was accessed by the number of TNC, % CD34 and cell multipotency from the expanded culture. During the course of development, by applying "rational media design"™ approach, we eliminated the serum, minimized the use of certain serum-derived components, such as transferrin, and identified non-animal derived components that can improve ex vivo HSC expansion. In addition, a clinical grade HSA was qualified and its concentration was optimized for the HSC medium. The final medium formulation was able to support HSC expansion, while maintaining its multipotent , as seen by their ability to generate different types of colonies in the colony-forming assay. When compared to two other commercially available serum-free media over multiple donor-derived HSCs, our final formation delivered a similar or higher specific fold increase of CD34+ HSCs over the benchmarks. This PRIME-XV® HSC Expansion XFSM formulation was then further optimized for large scale manufacturing under cGMP conditions. Overall, this new media offers a product of translational quality combined with high performance, making it ideal for clinical studies to applications.

F-2171

IN VITRO CARDIOTOXICITY AND NEUROTOXICITY ASSESSMENT OF ENVIRONMENTAL CHEMICALS USING ORGANOTYPIC HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MODELS

Chandy, Grischa and Sirenko, Oksana
Molecular Devices, Sunnyvale, CA, USA

Due to the increasing prevalence of neurological and cardiovascular disorders possibly related to exposure to environmental toxicants, there is an increasing need to develop reliable and efficient screening tools to identify environmental chemicals that could potentially affect human health. There is a great interest in using stem cell derived cell models for in vitro high-throughput quantitative assays that would allow for detecting the potential hazard of chemicals and prioritizing them for further testing. We developed several phenotypic screening assays testing neuronal and cardiac toxicity using imaging methods and induced pluripotent stem cell (iPSC)-derived cardiomyocytes and neurons. Human iPSC-derived cardiomyocytes and neurons were exposed to a number of known toxic compounds using concentration-responses and various lengths of time. We have tested a representative set of compounds that have been known to be associated with neurotoxicity or cardiotoxicity including pesticides, polycyclic aromatic hydrocarbons and flame retardants. Effects of compounds were assessed by high throughput automated imaging and image analysis. Effects on the development of neuronal networks were assessed by quantifying total neurite outgrowth, number of branches and processes, as well as cell viability. Effects on cardiomyocyte cellular and mitochondrial toxicity were assessed by using viability read-outs and mitochondrial depolarization probes. We have characterized a number of phenotypic read-outs that can be used for determining effective toxic concentrations and comparing the effects of different compounds. In addition we evaluated the feasibility of a combinatorial screening approach for functional and mechanistic toxicity profiling of environmental hazards. These studies demonstrate the utility of stem cell-based screening in identifying, ranking, and prioritizing compounds with cardiotoxic and neurotoxic potential for further in vivo testing.

F-2173

USE OF 3D CULTURED HUMAN IPSC-DERIVED HEPATOCYTES FOR LONG-TERM HEPATOTOXICITY STUDIES

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Drug-induced liver injury (DILI) or injury to the liver caused by prescription or non-prescription medications continues to be a growing public health problem and a challenge for drug development; compounded by new drug entities and the growing market for herbal and other non-traditional remedies. Most DILI is the result of unexpected responses to a medication or unforeseen long-term chronic damage. To test new drug entities for potential DILI, in vivo models remain the gold standard. However, these studies are costly, time-consuming and poor predictors of human toxicity due to the incorporation of mainly murine hepatocytes. Consequently, in vitro screens using primary hepatocytes are less costly, reduce animal exposure, and are more amenable to higher-throughput platforms. However, limitations such as high inter-individual variability, finite batch sizes and changes in cell morphology challenge this model. Human induced pluripotent stem cells (iPSC)-derived hepatocytes, by comparison, are a promising in vitro alternative to in vivo models by demonstrating primary tissue-like phenotype, high levels of consistency and unlimited availability. When performing toxicity studies, hepatocytes are repeatedly dosed with varying concentrations of a potential drug over multiple days to assess any cumulative effects. This poses particular challenges when incorporating two-dimensional (2D) cultured hepatocytes as cells rapidly de-differentiate and lose metabolic activity when cultured in this manner. Three-dimensional (3D) cell culture models allow cells to aggregate and retain functionality and communication networks

found in vivo. The favorable environment created by culturing in 3D allows performance of long-term dosing experiments that accurately analyze a potential drug's cumulative effects. Here we demonstrate the suitability of 3D cultured human iPSC-derived hepatocytes for use in hepatotoxicity studies. Hepatocyte spheroids were exposed to multiple concentrations of the DILI category I and III drugs talcapone, acetaminophen, and mitomycin C. Cell viability and CYP3A4 function was monitored, in addition to assessment of hepatocyte mitochondrial health, after short-term and long-term exposure to the drugs. Comparisons were also made to iPSC-derived hepatocytes cultured in 2D.

POSTER SESSION III-EVEN 19:00 – 20:00

PLACENTA AND UMBILICAL CORD DERIVED CELLS

F-1002

DOWN REGULATION OF AIMP3 VIA HEF1-HEY1 COMPLEX PLAYS A CRUCIAL ROLE IN REVERSING STEM CELL AGING

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Mesenchymal stem cells (MSCs) cultured in a common normoxia condition (21% O2) undergo senescence with reduced proliferation, differentiation capacity, and biological functions with passages whereas MSCs cultured in the hypoxia condition display prolonged proliferation with delayed senescence. Although the effect of the hypoxic condition on stemness or aging is well known, the anti-senescence mechanism is not clear. In this study, we investigated hypoxia (3% O2) mediated anti-aging/senescence mechanisms in human placenta mesenchymal stem cells (hpMSCs), recently shedding light on cell therapies due to easy acquisition without ethical issues. Compared to normoxia, the hypoxia culture condition increased proliferation capacity of hpMSCs without alterations in their characters over passage 15. The stem cell markers such as OCT4, NANOG, KLF-4, and c-MYC were expressed at the similar levels over the passages in the hypoxic condition. Hypoxia mediated delayed senescence was also indicated by several senescence-associated indicators: Sirtuin1 and Sirtuin 6, anti-aging markers, were maintained at the similar levels along the passages whereas p16INK4a, a senescence marker, was undetectable in the hypoxic condition. RNA sequence analysis showed that compared to the cells in normoxia, the hpMSCs in hypoxia upregulated proliferation, anabolic pathways, glycolysis, and hormonal response related genes, meanwhile protein aggregation and cell death related genes were downregulated. Among the suppressed genes in hypoxia, Aminoacyl-tRNA synthetase-interacting multifunctional protein 3 (AIMP3), known as a senescence inducer, was negatively regulated by both HIF1 and its interacting repressor partner, Hey1, in hpMSCs, and the reduced Hey-1 induced AIMP3 expression along with enhanced 16/INK4 expression and senescence. Consistent with in vitro results, MSCs from AIMP3 overexpressing mice appeared reduced stem cell proliferation and differentiation capacity and the neural stem cells in dentate gyrus were remarkably reduced in the transgenic mice. Here our study first demonstrated a novel mechanism where a novel HIF1-Hey1 interaction negatively regulated AIMP3 expression and AIMP3 mediated stem cell dysfunction, leading to delay the stem cell aging.

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F-1004

EFFECTS OF HUMAN UMBILICAL CORD MESENCHYMAL STROMAL CELLS ON THIRD DEGREE SKIN BURN WOUND HEALING IN RATS

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Most burns are caused by thermal injuries, and particularly serious thermal skin burns may result with death, albeit many therapeutic approaches. This study was designed to investigate the potential beneficial effects of the local transplantations of human umbilical cord mesenchymal stromal cells (hUCMSCs) in the third degree full thickness skin burns. After obtaining ethical approvals, male WistarAlbino rats (n=30) were divided into five groups as control, and hUCMSCs transplanted groups on day 0, 3, 7 and 30, after burn injury to evaluate the effects of cell applications in short or long term. hUC derived cells were immunophenotyped by flow cytometry using conventional MSC markers. Prior to the subcutaneous transplantation of these cells (4 million cells per wound), they were loaded with an in vivo tracker molecule, calceinAM, in order to analyze if these cells stay at the injection area or relocate to longdistance organs like lung. 45 days after burn injury; all groups were sacrificed and skin tissues were

obtained. The tissue integrity was examined by routine MalloryAzan staining. The antibodies produced against collagen typeI and III, VEGF, PECAM1, IL10, TSG6 or CK14 was used for immunohistochemistry analysis. Apoptosis was analyzed by TUNEL method. Three zones as a) coagulation zone (burnt area), b) stasis zone (healing area) and c) normal zone (uninjured area) were identified after MalloryAzan staining. While no significant difference was noted in the overall tissue organization between the groups, stasis zone was found to be significantly wider in hUCMSC treated ones when compared to others. While, no calceinAM loaded cells were noted in lung samples, they were found both in the coagulation and the stasis zones of the skin. Signal intensities and the distribution of collagen typeI and III, VEGF, PECAM1, IL10, TSG6 and CK14 signals were found to be comparable. The current study revealed that the local injection of hUCMSCs on the full thickness skin burns cause to increase in the width of the stasis zone, but do not exert any beneficial effect on the healing process as the skin appendages. On the other hand, since there is no difference between the groups in the analysed markers, we think that further studies are still required to explain the mechanism that hUCMSCs caused stasis zone wider.

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ADIPOSE, MUSCULOSKELETAL, AND CONNECTIVE TISSUE

F-1006

STEM CELL THERAPY FOR INTERVERTEBRAL DISC REGENERATION

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Mesenchymal stem cells (MSCs) have been considered to hold promise for treating intervertebral disc (IVD) degeneration. However, the different therapeutic efficacy of MSC has been a major problem and so far the derivation of MSCs for use in IVD degeneration has not been optimized. Preclinical study using Wharton's jelly-derived MSCs (WJ-MSCs) was performed in a rabbit model of IVD degeneration. We evaluated the effectiveness of human WJ-MSCs loaded in a cross-linked hyaluronic acid (XHA) scaffold for IVD regeneration according to the levels of transforming growth factor-β (TGF-β) receptor I/activin-like kinase receptor 5 (TβRI/ALK5) and II (TβRII) in a rabbit model. T2 MRI analysis after 12 weeks of transplantation showed significant restoration of disc water content when treated with MSChighTR loaded XHA as compared to the scaffold only and MSC-lowTR loaded XHA. In addition, morphological and histological analyses revealed the highest IVD regeneration at MSC-highTR loaded XHA transplanted groups. Taken together, clinical study using autologous adipose-derived stem cells are performing in patients with chronic discogenic back pain.

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F-1008

METABOLIC PROFILE AND SUSCEPTIBILITY TO ALDEHYDIC OVERLOAD IN A SKELETAL MUSCLE PRECURSOR MODEL OF MYOGENESIS

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Skeletal muscle accounts for ~30% of body weight. Muscle degeneration is related to several pathologies associated with metabolic disorders such as cancer, heart failure and diabetes, indicating that these cells must present an altered metabolic profile during commitment and differentiation. Skeletal muscle precursor cells have been used in several studies to understand the mechanisms of muscle regeneration, but their metabolic profile and aldehyde response remains elusive. Here, we characterized the metabolic profile of proliferating versus differentiated precursor cells of the skeletal musculature as well as their susceptibility to aldehyde overload. We used proliferating C2C12 pre-myoblasts plated and maintained in DMEM – containing 10% FBS in an atmosphere of 5% CO2 at 37°C. To differentiate cells into myotubes, they were cultivated for 5 days in serum restriction DMEM, supplemented with 2% horse serum (HS) and changed every 48 hours. XF24 extracellular flux analysis system was used to measure bioenergetic profile and API 4000 QTRAP mass spectrometer was used to measure intracellular aldehyde concentration. Protein levels and activity were measured by western blot and spectrophotometer, respectively. Proliferating cells present reduced mitochondrial complex V and aldehyde dehydrogenase 2 protein levels compared to differentiated cells, characterizing a decreased mitochondrial density under proliferation conditions. Indeed, proliferating cells present decreased basal oxygen consumption (440±21 vs. 714±13), lactate dehydrogenase (3.15±0.18 vs. 4.25±0.14) and aldehyde dehydrogenase 2 (0.056±0.003 vs. 0.093±0.006) activities compared to differentiated cells. This scenario

along with increased intracellular aldehyde levels characterizes an overall reduction of metabolic profile in proliferating cells. Finally, low amount of exogenous aldehydes (4-hydroxinonenal 50uM) significantly reduces oxygen consumption and induces death in proliferating, but not in differentiated cells. Therefore, we conclude that proliferating precursors have decreased mitochondrial metabolic profile (as expected) and are more susceptible to aldehydic overload compared to differentiated cells, leading us to propose a central role for cell fate in the susceptibility to aldehyde damage.

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F-1010

ISOLATION AND CULTURE OF ADIPOSE DERIVED STEM/STROMAL CELLS COLLECTED FROM POST-MORTEM ADIPOSE TISSUE

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Post-mortem tissues lose lost biological defense functions by various stimuli, such as stopping the oxygen supply or nutrient starvation due to the cessation of blood flow. Post-mortem livers, pancreases, and stomachs are invaded by its digestive enzymes. The invasion spreads to surrounding tissue over time. The speed of autolysis is affected not only by age and physique of the corpse, but also by the temperature and humidity of the environment. The corpse is given the encroachment in the resident bacteria, infecting bacteria from the outside, and animals. Stem cells from post-mortem tissues are used for experimentation. However the viability, pluripotency, and regenerative capacity are poorly understood. In addition, almost all of the studies have been limited to within 2 days after death. Adipose-derived mesenchymal stem/progenitor cells (ASCs) are prevalent in adipose tissue. ASCs can be isolated from excised adipose tissue by enzymatic digestion. ASCs have recently been given attention to clinical usage and drug discovery. ASCs not only function as tissue-specific progenitor cells but also are secrete various growth factors. Axillary adipose tissue is easy to harvest at autopsy, because of macroscopically less susceptible to injury, erosion, and autolysis. The innovative significance of this study is to determine the isolation and culture method of human ASCs from post-mortem adipose tissue. Frozen sections of adipose tissue were stained, and CD31-, CD34+, and CD45- human ASCs were observed by confocal microscopy. A heterogeneous cell mixture containing all cell types, except adipocytes, is extracted from axillary adipose tissue by Liberase enzyme treatment. The cell mixture was seeded and cultured to obtain human ASCs. It was confirmed with flow cytometry that almost all of the adhesively cultured cells are ASCs. It is possible to culture ASCs from post-mortem adipose tissue at least 7 days elapsed after death.

F-1012

ROBUST DERIVATION OF TRANSPLANTABLE SKELETAL MUSCLE PROGENITOR CELLS FROM HUMAN INDUCED PLURIPOTENT STEM (IPS) CELLS USING A STIRRING BIOREACTOR

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Human induced pluripotent stem cells (hiPSCs) are expected to be a cell source for cell therapy to treat devastating muscular dystrophies. To obtain skeletal muscle stem/progenitor cells from hiPSCs on a large scale, we modified EZ-sphere method by introducing continuous agitation of the culture with a bioreactor. Stirring bioreactor supported robust growth of myogenic spheres. hiPSC-derived myogenic cells were enriched in the CD57-negative, CD108-negative, CD271-positive, and ERBB3-positive cell fraction. FACS-sorted myogenic cells differentiated into myofibers in TA muscles of immune-deficient mdx mice after direct transplantation. In addition, hiPSC-derived myogenic cells efficiently fused with adult human myoblasts in vitro. Our results indicate that the EZ-sphere method with a bioreactor is useful to prepare transplantable cells for cell therapy of refractory muscle diseases.

F-1014

CRITICAL ROLE OF AN FK506-BINDING PROTEIN CONTROLLING MYOBLAST DIFFERENTIATION

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The molecular chaperons FK506-binding proteins (Fkbps) comprise one of three families of peptidyl prolyl isomerases, which promote the transition between cis- and trans-conformations of peptidyl prolyl bonds. Mouse Fkbp family is composed of at least 15 members, but the functions of the large family in cell proliferation and differentiation remain elusive. During myoblast differentiation, the cells need to exit the cell cycle before fusion and terminal differentiation to form myotubes. The clear distinction between proliferation and differentiation provides an ideal model with which to investigate the roles of Fkbps in these two cell biological events. We found that depletion of FkbpC in mouse myoblasts delayed the exit from the cell cycle and expression of myotube-specific genes, whereas its overexpression caused opposite effects. At a mechanistic level, our study revealed a crucial function of FkbpC in Cdk4 activation during myoblast proliferation. Cdk4 undergoes conformational changes in the HSP90/Cdc37/Cdk4 complex as a prerequisite for activation through binding to CyclinD1 accompanied by phosphorylation. Our results showed that FkbpC depletion released Cdk4 from the HSP90 complex, which increased the Cdk4/CyclinD1 complex in myoblasts and sustained high levels of phosphorylated Cdk4 and Rb during differentiation. These results explain the delayed cell cycle exit and differentiation in the depleted cells. In addition, after synchronizing the cell cycle of myoblasts we found dynamic changes of the amounts of FkbpC and Cdk4 in the HSP90 complex during the G1/S transition. Knockout mice of FkbpC demonstrated delayed muscle regeneration after chemical damage, providing an in vivo evidence for the essential role of FkbpC in muscle differentiation. Collectively, our study uncovered FkbpC's critical function as a novel switch regulating the transition from proliferation to differentiation through controlling one of the central regulators of proliferation, Cdk4.

F-1016

HEAT-SHOCK PROTEIN B8 AND ITS TARGETING MICRO-RNAS IN REGULATING DIFFERENTIATION CAPABILITY OF RAT DENTAL PULP STEM CELLS (DPSCS)

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Primary stem cells isolated from adult tissues need to be expanded to a sufficient quantity for therapeutic applications. Tissue derived adult stem cells (ASCs) often lose their differentiation capabilities or stemness during in vitro culture for expansion, and this is one of the major hurdles in applications of ASCs in tissue regeneration or tissue engineering. Elucidation of the factors causing the loss of differentiation could lead to development of methods to maintain differentiation capability during expansion of the ASCs. This study showed that dental pulp stem cells (DPSCs) lose their differentiation capability during in vitro expansion and heat-shock protein B8 (HspB8) is involved in maintaining the differentiation capability. Because micro-RNAs (miRNAs) have emerged as major regulators of stem cell fates, we screened expression of miRNAs that can potentially target HspB8 in DPSCs and found some of the HspB8-target miRNAs changed their expression during in vitro culture of the DPSCs. This study was conducted as described below. DPSCs were isolated from the first mandibular molars of rats. Cells were cultured in T-25 flasks to 90% confluency for passage. Cells at passages 3, 5, 7, 9 and 11 were collected for RNA isolation. Concurrently, cells of different passages were subjected to osteogenic induction to determine their differentiation capability. Expression of HspB8 in early and late passages of DPSCs was determined by real-time RT-PCR and western blot. Strong osteogenic differentiation was observed in passages 3 and 5 (P3 and P5) of the DPSCs. This capability was greatly reduced at passage 9 (P9) and completely lost at passage 11 (P11). Expression of HspB8 was down-regulated at passages 9 and 11 when the DPSCs lose differentiation potential. Knockdown of HspB8 expression in P3 and P5 DPSCs by siRNA resulted in loss of differentiation capability of the cells. Furthermore, HspB8-target miRNAs were predicted by TargetScan and expression of those miRNAs in early- and late-passage DPSCs was determined by Locked Nucleic Acid (LNA)-based Taqman stem-loop qRT-PCR. Let-7b, miR98, miR215, miR219 and miR295 were found to increase their expression in passages 9 or 11 as compared to passages 3 or 5. Functions of those miRNAs in regulating HspB8 and differentiation of DPSCs are under investigation.

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F-1018

IMPACT OF BONE-MARROW DERIVED MESENCHYMAL STEM CELL CONDITIONED MEDIA ON THE MIGRATION OF C2C12 MYOBLASTS: INFLUENCE OF OBESITY

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The growth promoting and regenerative capacity of bone marrow derived mesenchymal stem cells (BM-MSCs) is known to be mediated through paracrine functions and there are growing evidence to support the notion that BM-MSCs can promote skeletal muscle regeneration. The trophic abilities of endogenous BM-MSCs can however be altered in chronic inflammatory conditions. We therefore hypothesize that the pathogenesis of obesity-associated diabetes alters the secretome of BM-MSCs and that an altered cytokine secretion profile affects the ability of BM-MSCs to promote myoblast migration. Conditioned media (CM) was collected from primary BM-MSCs isolated from either healthy lean control (C57BL/6J) (CMcontrol) or obese pre-diabetic mice (B6.Cg-Lepob/J) (CMob/ob) and analysed for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, GM-CSF, IFNγ and TNFα concentrations. IL-6 (CMcontrol 369 ± 182 pg/mL; CMob/ob 48 ± 8 pg/mL) and TNFα (CMcontrol 8.8 ± 0.6 pg/mL; CMob/ob 5.5 ± 1.1 pg/mL) concentrations differed significantly ($p < 0.05$) between groups. CM was then used to treat C2C12 myoblasts and C3H/10T1/2 MSCs after infliction of an in vitro scratch injury and the rate of wound closure assessed. We demonstrate for the first time that the beneficial trophic effect of BM-MSCs on myoblast migration is compromised under obese pre-diabetic conditions. Our data furthermore indicate that BM-MSCs derived from obese pre-diabetic mice improved the migration of C3H/10T1/2 MSCs, suggesting a compensatory mechanism whereby dysfunctional BM-MSCs recruit additional MSCs. This study highlights the need for a better mechanistic understanding of BM-MSC trophic functions during muscle regeneration and the implications of pathological alteration in the microenvironment.

F-1020

CHONDROGENIC DIFFERENTIATION INDUCTION OF HUMAN ADIPOSE-DERIVED STEM CELLS BY CENTRIFUGAL GRAVITY

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Impaired cartilage cannot heal naturally. Currently, the most advanced therapy for defects in cartilage is the transplantation of chondrocytes differentiated from stem cells using cytokines. Unfortunately, cytokine-induced chondrogenic differentiation is costly, time-consuming, and is associated with a high risk of contamination during in vitro differentiation. However, biomechanical stimuli also serve as crucial regulatory factors for chondrogenesis. For example, mechanical stress can induce chondrogenic differentiation of stem cells, suggesting a potential therapeutic approach for repair of impaired cartilage. In this study, we demonstrated that centrifugal gravity (CG, 2400 × g), a mechanical stress easily applied by centrifugation, induced upregulation of SRY (sex determining region Y)-box 9 (SOX9) in adipose-derived stem cells (ASCs), causing them to express chondrogenic phenotypes. The centrifuged ASCs expressed higher levels of chondrogenic differentiation markers such as aggrecan (ACAN), collagen type 2 alpha 1 (COL2A1), and collagen type 1 (COL1), but lower levels of collagen type 10 (COL10), a marker of hypertrophic chondrocytes. In addition, a chondrogenic aggregate formation, a prerequisite for chondrogenesis, was observed in centrifuged ASCs.

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F-1022

THE ROLE OF PDGFRA+ AND PDGFRB+ CELLS IN REMODELING/REGENERATION OF THE MOUSE INJURED ROTATOR CUFF

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After sustaining a tear, the rotator cuff musculature often undergoes atrophy and fibroadipogenic degeneration that lead to significant activity related pain and decreased quality of life. While accumulation of fat tissue and fibrotic scarring are widely reported in human patients and animal experimental models, it is still unknown whether rotator cuff remodeling is accompanied by a transient phase of muscle regeneration prior to muscle atrophy, and what are the pathophysiological roles of muscle resident myogenic precursors after rotator cuff tears. PDGFRβ+ progenitors, distinct from myogenic satellite cells, reside in the muscle and include heterogeneous subsets of perivascular and interstitial cells. We used genetic fate tracing of GFP+PDGFRβ+ cells combined with a unique mouse model of irreversible rotator cuff tendon and nerve (TTDN) chronic injury to define the functional heterogeneity of PDGFRβ+ precursors. Histological analysis demonstrated that similarly to human injured muscle, murine muscle tissue undergoes significant fibroadipogenic remodeling and muscle atrophy following TTDN, which increased over time. While PDGFRβ+CD34+Pax7+α7-Integrin-PDGFRα- marked a distinct novel non-satellite cell subset with robust myo-regenerative capability, PDGFRβ+PDGFRα+ typified a fibroadipogenic cell subset. These subsets maintained their intrinsic characteristics in co-culture and after transplantation into injured muscle within 24 hours and 2 weeks following TTDN. Additionally, collagen type I promoted myogenesis by PDGFRβ+PDGFRα- cells and diminished that from satellite cells, coinciding with the observed stage-specific sequential remodeling of injured muscle. Together, a murine experimental model of massive rotator cuff tear, combined with PDGFRβ-Cre genetic fate tracing reveals the existence of rare myogenic PDGFRβ+Pax7+PDGFRα- progenitor cells that functionally differ from satellite cells, by maintaining their myogenic characteristics in the fibrotic environment. These findings support the development of innovative therapeutic strategies for transplantation of the non-fibrogenic, myogenic cell subset that will best regenerate injured muscle when delivered at a specific stage of remodeling.

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CARDIAC TISSUE

F-1026

VITAMIN D BINDING PROTEIN ENHANCES MATURATION OF CARDIOMYOCYTES DERIVED FROM HUMAN INDUCIBLE PLURIPOTENT STEM CELLS

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Cardiomyocytes differentiated from inducible pluripotent stem cells (iPSCs) remain in an immature state using current protocols, and do not have proper electromechanical integration when delivered to animal models. Previous studies have demonstrated that the extracellular matrix (ECM) may promote maturation of stem cell-derived cardiomyocytes. We sought to identify maturation factors found in the cardiac ECM. Freshly harvested bovine hearts were obtained from a slaughterhouse, decellularized, digested with trypsin, and fractionated by molecular weight using high performance liquid chromatography. Fractions of decellularized matrix were applied to iPSC-derived cardiomyocytes (iPSC-CMs) in culture for six days then screened for expression of selected genes of maturation (including CRYAB, ECHS, PDLIM5 and PYGM). The fraction with the highest expression levels of maturation genes was submitted for quantitative mass spectrometry to identify potential proteins associated with maturation. Through this screen, we identified vitamin D binding protein (VDBP) as a potential cardiomyocyte maturation factor. We cultured iPSC-CMs with 0-100 ng/mL of VDBP for 6 days and found a dose-dependent

increase in expression of selected genes of maturation, with a 2.5-fold increase of TNNT2 expression and 2-fold increase in MYH6 expression at 10 ng/ml VDBP ($p < 0.05$, n=4/group). Given that a major role for VDBP is to carry vitamin D3 to facilitate binding to vitamin D receptor (VDR), we also cultured iPSC-CMs with 0-100 nM 1,25(OH)2-D3 for 6 days and found a dose-dependent increase in expression of MYH6, TNNT2, and CACNA1c, suggesting that VDBP enhances iPSC-CM maturation by enhancing vitamin D3 delivery. Protein expression of TNNT2 was also increased following treatment with VDBP for 6 days by western analysis. The beneficial effects on gene expression in the presence of VDBP were eliminated when studied in a VDR knockout iPS line generated with CRISPR (clustered regularly interpaced short palindromic repeats) technology. These results identify VDBP and 1,25(OH)2-D3 as novel maturation factors for iPSC-CMs.

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F-1028

USING DRUG RESPONSES IN HUMAN IPSC-DERIVED CARDIAC MYOCYTES TO INFER MECHANISMS OF ACTION

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Human induced pluripotent stem cell derived cardiac myocytes (hiPSC-CMs) hold great promise for cell therapy, disease modeling and drug development. However, different differentiation protocols and cell maturation levels give rise to considerable variability in hiPSC-CMs physiological behaviors, both at baseline and under drug treatment. We hypothesized that a mathematical modeling approach that accounted for variability could overcome these limitations, and we developed a computational platform that predicts drug mechanisms of action on the basis of physiological recordings. To test the computational predictions, we profiled the effects of several ion channel blocking drugs on intracellular calcium (Ca2+) in iPSC-derived cardiac myocytes (hiPSC-CMs; day 30-35). Myocytes from several iPSC lines were tested, and Ca2+ transients (CaTs) were recorded in fluo-3 loaded cell monolayers before and after applying several concentrations of the following drugs: dofetilide, verapamil, nifedipine, procainimide, and ryanodine. Metrics quantified from the recordings included the spontaneous beating rate, CaT amplitude, CaT time-to-peak, and CaT duration at 50% and 90% (CaD50 and CaD90, respectively). We found that while the baseline physiology of the iPSC-CMs varied considerably from sample-to-sample, drug induced changes in CaT were highly reproducible. For example, dofetilide consistently reduced the spontaneous beating rate and prolonged CaD50 and CaD90. Nifedipine, in contrast, consistently reduced CaT amplitude, CaD50, and CaD90. These results, which are consistent with mathematical modeling predictions, suggest that drugs may induce specific physiological "signatures," and they support using physiological recordings to infer the mechanisms of action of drugs that affect cardiac myocytes.

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F-1030

WNT/ B-CATENIN SIGNALING RE-ACTIVATES PROLIFERATION OF ADULT CARDIOMYOCYTES

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The Wnt/β-catenin signaling pathway plays an important role in the development of second heart field (SHF Isl1+) that gives rise to the anterior heart field (AHF) cardiac progenitor cells (CPCs) for the formation of right ventricle, outflow tract (OFT) and portion of inflow tract (IFT). During early cardiogenesis, these AHF CPCs reside within the pharyngeal mesoderm (PM) that provides a microenvironment for them to receive signals that direct their cell fates. Here, N-cadherin, which is weakly expressed by CPCs plays a significant role by promoting the adhesion of CPCs within AHF and maintaining high Wnt signaling activity through the regulation of β-catenin levels in the cytoplasm to activate Wnt target genes for cardioproliferation and to prevent premature differentiation of CPCs. On the contrary, strong expression of N-cadherin was observed throughout adult myocardium, suggesting a down-regulation of Wnt signaling as β catenin molecules are sequestered at the cell membrane. Henceforth, we investigate if reactivation of Wnt signaling is able to enhance

cellular proliferation of adult cardiomyocytes. Indeed, either disruption of N-cadherin signaling or inhibition of GSK were shown to increase proliferation of both adult mouse and human ES derived cardiomyocytes, providing potential therapeutic options to treat patients with myocardial infarction.

F-1032

IDENTIFYING LATE-ONSET FABRY CARDIOMYOPATHY WITH THE GLA IVS4+919G>A MUTATION IN PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

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Fabry disease (FD) is an inherited lysosomal storage disorder caused by α-galactosidase A (α-Gal A) deficiency. A surprisingly high incidence of a cardiac variant GLA mutation, IVS4+919G>A, has been reported in Taiwan in adult patients with idiopathic hypertrophic cardiomyopathy and in newborn infants. However, the underlying pathogenesis of FD cardiomyopathy in patients with the IVS4+919G>A genotype is unknown. We generated FD-specific induced pluripotent stem cells (FD-iPSCs) from ten IVS4+919G>A patients with hypertrophic cardiomyopathy with a lysosomal accumulation of globotriaosylceramide. Using FD-iPSC-differentiated cardiomyocytes (FD-iPSC-CMs) as a disease model, we showed that FD-iPSC-CMs carrying the IVS4+919G>A mutation recapitulated several FD cardiomyopathy-specific characteristics, including decreased GLA expression/activity, cellular hypertrophy, contractile-dysregulated electrophysiology, and lysosomal accumulation of globotriaosylceramide. Proteomic analysis revealed that two arachidonate lipoxygenases, Alox12 and Alox15, were substantially upregulated in FD-iPSC-CMs compared with control-CMs. Analysis with ELISA showed an increased secretion of 12/15- hydroxyeicosatetraenoic acid (HETE), the metabolites of Alox12/Alox15, in the culture medium of FD-iPSC-CMs. The addition of glycosphingolipid-Gb3 significantly induced Alox12/Alox15 expression and 12/15-HETE secretion in FD-iPSC-CMs compared with control-CMs. In the IVS4+919G>A mutation patients, hypertrophic myocardium exhibited high expression levels of Alox12/Alox15, as detected by immunohistochemistry in cardiac biopsy samples, and high serum 12/15-HETE levels compared with the age- and sex-matched controls. Our findings demonstrated that FD-iPSC-CMs recapitulate the hallmarks of cardiac abnormalities in FD patients with the IVS4+919G>A genotype and may represent a high-throughput platform for investigating the mechanism of FD cardiomyopathy. A potential remedy targeting the Alox12/Alox15 network should warrant investigation of further novel biomarkers and therapeutic strategies against this inherited disease.

F-1034

CRYOPRESERVATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES USING PROGRAMMED FREEZING WITH AN OSCILLATING MAGNETIC FIELD

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Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) are promising sources applicable to therapy of cardiovascular diseases, drug discovery and cardiotoxicity screening. Efficient cryopreservation of hiPSC-CMs is essential to broad dissemination of the cell technology in an industrialized fashion. Nevertheless, survival rates after thawing of cryopreserved hiPSC-CMs are currently low, generally up to 60%. Here we report an efficient hiPSC-CM cryopreservation method that shows more than 85% survival rate after thawing. Cell Alive System (CAS), which combines a programmed freezer with an oscillating magnetic field, is a freezing technology that brought innovative quality improvement in frozen food, and recently in cells. We optimized settings of magnetic field and current frequency for cardiomyocytes. With a unique combination of them, hiPSC-CMs were efficiently survived after the frozen-thawed process. The survived hiPSC-CMs showed no apparent changes in features as cardiomyocytes including heart rates or mRNA expression of cardiac markers, TNNT2, MYL2 and MYH6. These results suggest that the CAS freezer is a novel useful method for the cryopreservation of hiPSC-CMs, which would largely contribute to industrialization of hiPSC-CM-based technologies.

F-1036

IDENTIFICATION OF CD82-POSITIVE HUMAN CARDIOMYOCYTE-FATED PROGENITORS

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Currently, human cardiac progenitors that have their fate restricted to cardiomyocytes (CMs) have not been identified. Although cardiovascular progenitors differentiate into CMs with high efficiency in optimized cell culture conditions in vitro, they show deviated differentiation into non-CM populations such as

vascular or other stromal cell lineages in non-specific culture conditions and after transplantation in vivo. So, identification of CM-fated progenitors (CFPs) that cell-autonomously and exclusively differentiate into CMs even in diverse microenvironments in vitro and in vivo is anticipated. To identify CFPs, we examined the differentiation spectrum of KDR+PDGFRα+ (KP) cells using a stepwise differentiation protocol for human iPSCs to CMs based on a high density monolayer culture (Modified DD protocol. PLoS One, 2011) and found the fate decision of KP cells towards CMs occurred during day4 to 5 of differentiation. By comparing global gene expression profiles, we detected CD82/KAI1 as a top candidate gene that specifically upregulated during the period. CD82 was transiently expressed in late-stage mesodermal cells. Purified CD82+ cells specifically gave rise to CMs even in non-specific in vitro culture condition with serum and in vivo after transplantation to sub-renal space or injured hearts in mice, indicating that CD82+ cells are cell-autonomous CFPs. To examine the functional role of CD82, we performed CD82 overexpression experiments. Overexpression of CD82 in hiPSCs increased exosome secretion containing β-catenin and reduced nuclear β-catenin protein to cause Wnt signal inhibition, suggesting that CD82 is involved in the fate restriction to CMs through exosome-mediated Wnt inhibition. This study should contribute to novel cardiac regeneration strategies and the understanding of CM differentiation mechanisms.

Funding Source: This work was supported by research grants from the Ministry of Health, Labor, and Welfare, Japan and Uehara Life Science.

ENDOTHELIAL CELLS AND HEMANGIOBLASTS

F-1038

NOX4 NADPH OXIDASE SIGNALLING PLAYS A CRITICAL ROLE IN POSTISCHAEMIC REVASCULARISATION MEDIATED BY ENDOTHELIAL COLONY-FORMING CELLS

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Endothelial colony-forming cells (ECFCs), a class of endothelial progenitor cell (EPC), hold great therapeutic potential for ischaemic disease. Emerging evidence supports a key role for reactive oxygen species and NADPH oxidases in the underlying angiogenic processes of EPCs and other endothelial cells, where Nox4 is the major expressed isoform. To study the influence of Nox4 on the proangiogenic function of ECFCs, cells from umbilical cord blood were assessed (1) in vitro under basal conditions (DMEM or EGM2), with pro-oxidative stimuli or modified Nox4 expression, using migration and tubulogenesis assays, and (2) in vivo using an established model of experimental hindlimb ischaemia in SCID mice to assess revascularisation. PMA (100nM, 16h) increased cell migration (wound closure: control 23.3.±7.9, PMA 51.1±19.3 arbitrary units (au); n=6, P < 0.001) and tubulogenesis (branch length: control 6.8±0.9, PMA 8.4±1.4 au; n=6, P < 0.05), which was inhibited by the pan-Nox inhibitor VAS2870, while basal tube formation was reduced by VAS2870 (branch length: control 6.8±0.8, VAS2870 3.6±2.0 au; n=6, P < 0.01), highlighting Nox dependency. Complementary RT-PCR analysis found Nox4 to be the only isoform transcribed in ECFCs at biologically relevant levels, with enhanced expression following PMA treatment (mRNA: control 7.0±0.6, PMA 16.6±1.2 au relative to HSP90AB1; n=9, P < 0.001). Further, ECFC migration was reduced by siRNA knockdown of Nox4 (wound closure: control siRNA 173.7±18.3, Nox4 siRNA 96.1±23.3 au; n=9, P < 0.001), as was tubulogenesis (branch length: control siRNA 6.9±1.2, Nox4 siRNA 4.6±0.7 au; n=9, P < 0.001), highlighting a key role for Nox4 NADPH oxidase. Notably, in a murine model of ischaemia, administration of ECFCs enhanced revascularisation assessed by laser Doppler 7 days after femoral artery ligation (PBS 0.32±0.08, ECFC 0.71±0.27 ischaemic/control limb ratio; n=6, P < 0.05), not seen following Nox4 knockdown (control siRNA 0.71±0.27, Nox4 siRNA 0.39±0.17 ischaemic/control limb ratio; n=6, P < 0.05), also highlighting a key role for Nox4 in vivo. Taken together, ECFC proangiogenic function appears enhanced by Nox4 signalling, and critically-dependent on Nox4 both in vitro and in vivo, a finding which may have significant implications for ECFC-based cytotherapies for ischaemic disease.

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F-1040

STEM CELLS AND NEUROVASCULAR DYSFUNCTION: EVALUATING THE FUNCTIONAL CONSEQUENCES OF VARIANTS

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"The gold standard for diagnosis of Parkinson's disease has been the presence of SNpc [substantia nigra pars compacta] degeneration and Lewy pathology at post-mortem pathological examination." (Kalia 2015) We hypothesize the neurovascular compartment plays a critical role in neurodegeneration. Parkinson's disease (PD) is diagnosed using clinical features (Frasier 2014)

and there is no test for definitive diagnosis of PD at early stages of the disease (Kalia 2015). Biomarkers are critically needed for diagnosing patients with PD, as well as the subclinical PD cohort in whom early interventions could be made, preserving neurologic function. To identify a connection between putative biomarkers and clinical manifestations (such as degeneration, inflammation, structural alterations) we propose to evaluate the potential role of pericyte deficiency/loss ("pericyte ghosts") in progressive vascular degeneration and blood brain barrier (BBB) disruption (Zlokovic 2011), with comprehensive analysis of exome sequencing data (PPMI Research Biomarkers - from whole-blood extracted DNA samples), for genetic variants and the evaluation of the functional consequences of the variants (classified as pathogenic) in progenitor subsets identified in the neurovascular compartment/BBB; to explore a vasculotoxic perspective of epithelial–mesenchymal transition (EMT) vs mesenchymal–epithelial transition (MET); to identify potential biomarkers (such as cytokines and inflammatory factors) by single-cell analysis and cytometry assay development; and to perform ancillary ocular imaging studies to detect ocular manifestations with non-invasive diagnostic identification of ocular abnormalities/pathology. Preliminary data will be presented, including 1) genomic and epigenomic signatures; and 2) potential signaling and regulatory pathways. The results of the study can provide insight on the potential critical role of neurovascular dysfunction, identify the molecular mechanisms that relate to vascular stability (von Tell 2006) in neurodegenerative disorders and other pathological conditions/vascular dysfunction (tumor vasculature/retinopathy), and lead to promising therapeutic discoveries.

HEMATOPOIESIS/IMMUNOLOGY

F-1042

LIVE ANIMAL IN VIVO TRACKING OF NATIVE LONG-TERM HEMATOPOIETIC STEM CELLS USING A NOVEL LT-HSC EXCLUSIVE ANIMAL MODEL

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To fully understand the native perplexity of long-term hematopoietic stem cell (LT-HSC) behavior it is of great significance to be able to perform live-animal imaging. As the current identification and isolation of LT-HSCs is carried out via multi-parametric flow cytometry sorting (FACS), such real-time HSC tracking is not feasible. Moreover, recent mouse models that have been described to track with LT-HSCs require additional surface markers to restrict their expression to the LT-HSC compartment hence limiting their use for live animal imaging. Thus, it is crucial to generate an HSC-specific reporter mouse model in which LT-HSCs can be tracked in real-time. This type of model will enable live animal imaging studies to assess the behavior and dynamic cellular interaction of native LT-HSCs with nearby niche cells. To that end, we have developed a novel binary transgenic mouse model that exclusively marks LT-HSCs and can be used for live animal calvarium bone marrow (BM) imaging via intravital microscopy. Extensive characterization via FACS analysis showed that labeled LT-HSCs represent a rare fraction of previously described LT-HSCs and are exclusively found within the BM stem cell compartment. Low cell number transplantation experiments of labeled cells demonstrated self-renewal and multi-lineage differentiation capacity. Labeled LT-HSCs were found to be highly dormant via cell cycle analysis and transcriptionally indistinguishable from SLAM cells via single cell transcriptional profiling. Finally, we utilized live animal in vivo calvarium BM imaging to dissect their location and behavior. Our data demonstrate a sinusoidal niche for LT-HSCs in the calvarium that was confirmed in corresponding fixed femur bone sections. In addition, we successfully performed long-term time-lapse imaging experiments to determine the LT-HSC mobility and motility changes under normal and treatment conditions. These experiments revealed striking differences between steady- and mobilization-state and will be presented during the meeting. Overall, this work provides a novel LT-HSC marking animal tool that enables the assessment of native LT-HSCs behavior and dynamics in real time.

F-1044

THE MUTUAL EVL/MIR-342 GENOMIC LOCUS REGULATES LYMPHOID AND MYELOID DIFFERENTIATION PROGRAMS

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Extrinsic and intrinsic factors tightly control HSC regulation to adapt the production of blood cells to the organism's need. During the last years, it has become clear that non-coding genes like miRNAs contribute to hematopoietic differentiation programs. However, the molecular interplay between intronic miRNAs and their host genes is not yet fully understood. By investigating

the clonal dynamics of genetically corrected peripheral blood (PB) in ten Wiskott-Aldrich-syndrome patients in a clinical gene therapy trial, we identified the Evl/miR-342 gene locus as hotspot for therapeutic vector insertions suggesting its long-term activity in human hematopoiesis. To study the role of this candidate locus in hematopoiesis, we overexpressed EVL and miR-342 lentivirally in murine primary LSK (lin-Sca1+ckit+) cells. Overexpression (OE) of Evl led to a 7.5-fold increase of preB-cell colonies as compared to mock-transduced LSK cells, and a significantly increased donor-derived B-cell frequency within the spleens of primary recipient mice as well as PB after secondary transplantation. By contrast, miR-342 OE led to a >2.3-fold number of myeloid colonies in vitro and a 2.4-fold increased number of myeloid progenitor-derived CFU-S in vivo. In line with this, global gene expression profiling revealed a profound deregulation of canonical pathways essential for the development of B-cells upon Evl, and myeloid development upon miR-342 OE. Both, the in-silico and experimental identification of miR-342 targets indicate that miR-342 suppresses lymphopoiesis. As our results point to an essential role of the Evl/miRNA-342 gene locus in lymphoid or myeloid lineage determination, we assessed its deregulation in human hematopoietic malignancies. Strikingly, the publicly available gene expression dataset of 2096 leukemia samples embedded in the MILE study demonstrated a significantly increased Evl expression in all lymphoid leukemias compared to healthy BM or myeloid malignancies. In summary, our data show that one common gene locus regulates distinct hematopoietic differentiation programs depending on the gene product expressed. While the protein-coding gene Evl drives B-cell lymphopoiesis, its intronic miR-342 promotes myeloid differentiation.

F-1046

DISTINCT ROLES FOR MATRIX METALLOPROTEINASES 2 AND 9 IN EMBRYONIC HEMATOPOIETIC STEM CELL PRODUCTION, LOCALIZATION, AND MATURATION

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In the zebrafish embryo, hematopoietic stem/progenitor cells (HSPCs) are specified from hemogenic endothelium in the ventral wall of the dorsal aorta (VDA). While many factors controlling HSPC specification and production have been discovered, the mechanisms underlying the physical egress, "budding", and movement of HSPCs to secondary niches are not understood. Matrix metalloproteinases (MMPs) are a group of extracellular matrix (ECM)-modifying enzymes that regulate cell interactions, migration and signaling. The gelatinases, MMP2 and MMP9, are expressed in the hematopoietic tissues and regulated by inflammatory signals, such as prostaglandin-E2. Chemical and genetic inhibition of MMP2 during HSPC specification and emergence led to an abnormal expression pattern of the conserved HSC markers runx1 and cmyb in the VDA. FACS analysis of embryo sections revealed a significant accumulation of HSPCs in the VDA compared to controls, concomitant with decreased HSPC number in the caudal hematopoietic tissue (CHT; fetal liver equivalent). Additionally, lymphoid progenitor number was decreased in the thymus, as determined by WISH and FACS analysis, suggesting delayed HSPC colonization of adult HSC niches. Live confocal imaging further documented appropriate HSPC specification, but failures in budding in the absence of MMP2 activity. Significantly, this phenotype was not seen in embryos deficient in the MMP2 substrate fibronectin (fn1 mutants), indicating that MMP2 digests fibronectin-rich ECM in the VDA to facilitate extravasation. In contrast, MMP9 was dispensable for HSPC specification and budding, but loss at later timepoints caused a significant accumulation of HSPCs in the CHT at the expense of thymic seeding. This MMP9-associated migration phenotype was mimicked by overexpression of C-X-C motif chemokine ligand 12 (cxcl12), a known proteolytic target of MMP9. Furthermore, this MMP9-dependent HSPC aggregation could be blocked by cxcl12 knockdown, suggesting that MMP9 controls colonization of secondary sites of hematopoiesis by regulating chemokine signaling. In sum, our findings indicate that MMP2 and MMP9 play distinct but complementary roles in controlling HSPC movements within and between embryonic niches via proteolytic cleavage of ECM and regulation of chemokine networks, respectively.

F-1048

EMPOWER THE STEMNESS IN NORMAL AND LEUKEMIC HEMATOPOIESIS BY THE HOXB CLUSTER REGULATED VIA THE RETINOID-DEPENDENT ENHANCER

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Hox homeobox genes have been shown in the adult hematopoietic system to be critical for the stem cell property or stemness, and reacquired Hox gene expression in progenitors is a distinguished feature of leukemia stem cells. Besides, ectopic expression of Hox genes endows mouse embryonic stem cells (ESCs) with definitive hematopoietic stem cells (HSCs) features, and defective Hox gene expression constitutes a barrier for induction of human ESCs into functional HSCs. However, whether and how Hox genes are regulated as a cluster by the genetic and epigenetic regulatory networks remain largely unknown. Here we performed transcriptome, DNA methylome, and H3K27ac ChIP-seq profiles in HSCs, and identified a retinoid-dependent enhancer, distal element of retinoic acid response element (DERARE), which orchestrates global regulation of Hoxb cluster expression in HSCs. Deletion of the DERARE, or retinoic acid receptor (RAR) that binds to DERARE, abrogated Hoxb cluster expression and downstream non-canonical Wnt signaling, resulting in loss of HSC quiescence and long-term function. Mechanistically, DERARE is a methylation-sensitive enhancer, whose methylation is mediated by Dnmt3a. Finally, acute myeloid leukemia (AML) patients with DNMT3A mutations had low DERARE methylation, which correlated with elevated HOXB expression and adverse outcomes. Our findings demonstrate the fundamental roles of the retinoid-dependent and methylation-sensitive enhancer DERARE in integrating retinoid signaling to control the maintenance of HSCs and prevention of leukemogenesis by regulation of Hoxb cluster genes.

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F-1050

INTERFERON GAMMA-STIMULATED MACROPHAGES RESTRICT PLATELET-PRIMED HEMATOPOIETIC STEM CELLS AND LEAD TO THROMBOCYTOPENIA AND MORTALITY IN SEVERE APLASTIC ANEMIA

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Interferon gamma (IFNγ) negatively regulates hematopoietic stem cell (HSC) number and function and is associated with bone marrow (BM) failure syndromes in mouse and man. We previously identified the BM resident macrophage (MΦ) niche as a key sensor of IFNγ that negatively regulates HSCs during intracellular bacterial infection. Using a lymphocyte infusion-based murine model of severe aplastic anemia (SAA), we demonstrate that IFNγ drives disease in a MΦ-dependent manner. IFNγ signaling was essential for preserving BM resident MΦ populations, despite extreme hypocelluarity, loss of other myeloid cells, and HSC loss. Methods to deplete MΦs or abrogate IFNγ-signaling in MΦ-lineage cells during SAA rescued HSC loss without perturbing other elements of SAA pathology, including inflammatory cytokine production, T cell recruitment to the BM, and T cell activation. MΦ depletion, or abrogating IFNγ signaling in MΦs, during SAA induced the emergence of a CD41hi HSC population, which correlated with robust platelet production, increased megakaryocytes in the BM, and reduced SAA-associated mortality. HSCs from MΦ-depleted donors demonstrated enhanced platelet production when transplanted, whereas in vivo platelet label retention was equivalent to control SAA mice. These data indicate that MΦs restrict emergency thrombopoiesis by impairing platelet-primed HSCs, rather than by promoting platelet clearance. Since megakaryocytes serve as HSC niches in the BM, we reason that MΦs additionally impair HSCs by reducing supportive niche cells. We further identify that BM MΦs express podoplanin (PDPN) during SAA, and blockade of PDPN rescued HSC loss, thrombocytopenia, and death. Our data demonstrate a novel role for MΦs in impairing the HSC pool during SAA, and reveal mechanistic insight as to how IFNγ drives hematopoietic failure. Modulation of the BM resident MΦ niche or PDPN signaling warrants further investigation as a potential treatment for SAA patients.

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F-1052

MODELING X-LINKED SIDEROBLASTIC ANEMIA WITH PATIENT DERIVED HUMAN INDUCED PLURIPOTENT STEM CELLS

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Sideroblastic anemias are a heterogeneous group of inherited and acquired disorders. The most common type of hereditary sideroblastic anemia is X-linked sideroblastic anemia (XLSA) associated with mutations in the erythroid-specific δ-aminolevulinic acid synthase (ALAS2) gene. ALAS2 encodes the enzyme that catalyzes the first steps in heme synthetic pathway in erythroid cells. Heme synthesis starts from the step that glycine and succinyl CoA is polymerized and δ-aminolevulinic acid (ALA) is synthesized in the mitochondria. This step requires pyridoxal 5'-phosphate (PLP) as a cofactor. Though the treatment with PLP is effective for a small part of patients with XLSA, there are no other effective treatments. We attempted to generate induced pluripotent stem cells (iPSCs) from peripheral blood mononuclear cells (PBMCs) from an XLSA female patient, who harbored the heterozygous mutation (R227C) in the ALAS2 gene, utilizing episomal methods. She showed severe anemia and skewed X-chromosome inactivation in PBMCs with preferential inactivation of the X chromosome carrying the normal ALAS2 gene, indicating a condition associated with unbalanced lyonization. We successfully established both iPSC lines with the active mutant ALAS2 allele and those with the active wild-type ALAS2 allele. We assessed hematopoietic differentiation potential of these two types of iPSC lines using the embryoid body formation. Differentiation into hematopoietic progenitor cells (HPCs) was comparable in two groups. However, further differentiation in erythroid culture was significantly impaired in all tested iPSC lines harboring the active mutant ALAS2 allele compared with those harboring the active wild-type ALAS2 allele. Only mutant ALAS2 expression was observed in the erythroid cells differentiated from the iPS cells harboring the active mutant ALAS2 allele. Furthermore, administration of ALA to HPCs differentiated from iPSC clones harboring the active mutant ALAS2 allele greatly improved the erythroid differentiation efficiency. Our data suggests that this disease-specific iPS cell system is useful for studying molecular etiology of XLSA and for identifying novel therapeutic strategies for this disease.

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F-1054

ENHANCED IN VITRO PLATELET PRODUCTION THROUGH SRC FAMILY KINASE INHIBITION

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Approximately 7,000 units of donated platelets are required daily in the US to treat patients suffering from thrombocytopenia. Platelet counts below 20,000/ul increase the risk of life-threatening bleeding. However the lengthy donation process and short platelet shelf life results in chronic platelet shortages. One strategy to overcome these challenges is to generate donor-free platelets in vitro. While current in vitro methods can produce mature megakaryocytes (Mks), the platelet producing cell, platelet production remains inefficient. In vivo, each Mk releases approximately 3,000-5,000 platelets. However, in vitro systems generate on average ~30 platelets/Mk at best. The reasons for poor platelet production are not well understood, but may reflect differences in cell signaling and Mk terminal maturation in vitro versus in vivo. The megakaryocyte transcription factors RUNX1, GATA1, and FLI1 assemble into a large enhancesome complex that promotes terminal Mk maturation and platelet formation. The phosphorylation state of proteins in this complex regulates their assembly and transcriptional activity. Src family kinase (SFK) mediated tyrosine phosphorylation of RUNX1 inhibits its interaction with GATA1 and impairs Mk maturation. We therefore tested the ability of SFK inhibitors (PP2, Dasatanib, and SU6656) to enhance Mk maturation and platelet release in human CD34+ cells. Cells treated with PP2 and SU6656 exhibit enhanced markers of Mk maturation including higher ploidy and increased CD42b expression, and release more platelets into the culture compared to vehicle treated cells. Future work will focus on manipulating Mk transcriptional regulatory proteins in human induced pluripotent stem cells (hIPSCs) and characterizing the quality of the derived platelets. This will ultimately contribute to establishing an in vitro source of platelets for clinical use.

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F-1056

INFLAMMATORY REGULATION OF HEMATOPOIETIC STEM CELL FORMATION AND FUNCTION IN THE NORMAL AND HYPERGLYCEMIC VERTEBRATE EMBRYO

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Hematopoietic stem cells (HSCs) arise from a specialized subset of endothelial cells in the embryonic aorta. Once formed, HSCs circulate and colonize secondary niches, where they proliferate and differentiate to maintain lifelong hematopoiesis. Although many intrinsic factors regulating HSCs have been revealed, there is an emerging role for cell-extrinsic regulation of both HSC formation and long-term function. We previously reported that acute exposure to excess glucose accelerated the onset and magnitude of HSC formation in the zebrafish embryo. Additionally, we, and others, recently demonstrated that locally produced pro-inflammatory cytokines stimulate embryonic HSC production across vertebrates. As hyperglycemia is known to promote inflammation, we explored the hypothesis that hyperglycemia augments inflammatory signaling during HSC formation to influence output and cell fate. In support of this possibility, exposure to elevated glucose levels during HSC specification (12-36 hours post fertilization (hpf)) increased expression of several pro-inflammatory cytokines and receptors. To determine long-term effects of glucose exposure, embryos were treated during the entire window of developmental hematopoiesis (24-120hpf) and during maturation and seeding of secondary organs (72-120hpf). In addition to increased numbers of HSCs, sustained hyperglycemia (24-120hpf) increased expression levels of several inflammatory cytokines: ifng, ifn-phi, il1b, il6, tnfa and/or their receptors. Interestingly, glucose exposure from 72-120hpf increased mpo expression and numbers of Mpo+ myeloid cells enumerated by flow cytometry; conversely, rag1 expression, indicative of lymphoid commitment, was reduced in the thymus, suggesting altered HSC function or maintenance. Morpholino-mediated knockdown studies suggest roles for IFNg, TNFa and IL-1b downstream of metabolic stimulation in regulating HSC lineage choice. Ongoing investigation of the mechanisms connecting hyperglycemia and inflammation implicates both insulin signaling and inflammasome activation in promoting lineage skewing. As offspring of diabetic mothers exhibit a higher risk of childhood leukemia, understanding the consequences of metabolic alterations on HSC function is of clinical interest.

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F-1058

ROLE OF ARS2 IN PROLIFERATION AND DIFFERENTIATION CAPABILITIES OF MOUSE HEMATOPOIETIC STEM CELLS

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Hematopoietic Stem Cells (HSCs) generate all blood cell lineages while maintaining their own small population, requiring a delicate balance between self-renewal and differentiation. This balance relies on tight regulation of gene expression programs. Ars2 was found to coordinate gene expression with cellular proliferation through its co-transcriptional association with the 7-methylguanosine cap-binding complex. Moreover, Ars2 is required for maintenance of normal bone marrow cellularity, as knockout of Ars2 in adult mice led to marked bone marrow hypoplasia and reduction of stem and progenitor LSK (lin-, Sca+, c-Kit+) cells. In this study, we set out to test the hypothesis that Ars2 is required for self-renewal of bone marrow progenitor cells due to its ability to coordinate gene expression with proliferation. To eliminate effects of Ars2 depletion on bone marrow stromal cells, we developed a chimeric mouse model in which bone marrow cells from either Ars2f/f mice or Ars2f/f mice expressing tamoxifen-inducible Cre recombinase (Cre-ERT2) were used to reconstitute lethally irradiated C57BL\6 mice. Surprisingly, we found an increase in Ki67+ cells in Ars2f/f, CreERT2 chimeric mice following tamoxifen injection, suggesting that Ars2 is not required for proliferation of bone marrow cells. Flow cytometry analysis of Ars2 knockout bone marrow found an increased percentage of LSK cells, yet a decrease in long-term (LT) HSCs (lin-, Sca+, c-Kit+, CD34- CD150+ CD48-). This suggested that bone marrow progenitor sub-populations may be differentially sensitive to Ars2 depletion. To explore this possibility further, we performed single cell colony formation assays to assess the differentiation capability of Ars2-floxed versus Ars2-knockout LT-HSCs. We obtained several large colonies from singly-sorted Ars2-floxed LT-HSCs consisting of morphologically mature cells of all hematopoietic lineages. In contrast, singly-sorted Ars2-knockout LT-HSCs produced small, undifferentiated colonies. These findings expose Ars2 as a crucial factor in maintaining HSC self-renewal and differentiation capacity. Future studies will address the molecular details of how HSCs utilize Ars2 to execute

gene expression programs that facilitate their self-renewal and/or differentiation.

Funding Source: NIH: R00 CA175189

PANCREAS, LIVER, KIDNEY

F-1062

ESTABLISHMENT OF A NON-HUMAN PRIMATE MODEL OF CHRONIC LIVER INJURY TOWARD CELL THERAPY USING HUMAN IPSC-DERIVED HEPATOCYTE-LIKE CELLS

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Orthotopic liver transplantation (OLT) is prevalent all over the world as a radical treatment for liver failure and inherited metabolic liver diseases. However, OLT is limited by the severe scarcity of donor livers and many patients are suffering from these liver diseases without having the operation. Since the establishment of human iPSCs in 2007, cell transplantation using hepatocyte-like cells (HLCs) derived from human iPSCs has been expected as the bridging therapy or the alternative therapy to whole organ liver transplantation. Towards clinical applications preclinical tests using non-human primates are essential to securing safety and efficacy of the transplantation therapy. However, there are no reports so far describing non-human primate models of chronic liver injury. In this study, we succeeded in establishing drug-induced chronic liver injury models in monkeys (Macaca fascicularis). Furthermore, we transplanted human iPSC-derived HLCs into the chronic damaged liver via the portal vein and confirmed engraftment of transplanted human HLCs. The non-human primate chronic liver injury model we established will be a promising and powerful tool for technological development of cell transplantation and preclinical tests using human iPSC-derived HLCs.

F-1064

TRANSCRIPTION FACTORS AND MEDIUM SUITABLE FOR INITIATING THE DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO THE HEPATOCYTE LINEAGE

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Transcription factors and culture media were investigated to determine the condition to initiate the differentiation of human induced pluripotent stem (iPS) cells most efficiently. The expression of genes in human adult liver was compared with that in 201B7 cells (iPS cells) using cDNA microarray analysis. Episomal plasmids expressing transcription factors were constructed. 201B7 cells were transfected with the episomal plasmids and cultured in ReproFF (feeder-free media maintaining pluripotency), Leibovitz-15 (L15), William's E (WE), or Dulbecco's Modified Eagle Medium/Nutrient F-12 Ham (DF12) for seven days. RNA was isolated and subjected to real-time quantitative PCR to analyze the expression of alpha-feto protein (AFP) and albumin. cDNA microarray analysis revealed 16 transcription factors that were upregulated in human adult liver relative to that in 201B7 cells. Episomal plasmids expressing these 16 genes were transfected into 201B7 cells. CCAAT/enhancer binding protein alpha (CEBPA), CCAAT/enhancer binding protein beta (CEBPB), forkhead box A1 (FOXA1), and forkhead box A3 (FOXA3) up-regulated AFP and down-regulated Nanog. These four genes were further analyzed. The expression of AFP and albumin was the highest in 201B7 cells transfected with the combination of CEBPA, CEBPB, FOXA1, and FOXA3 and cultured in WE. The combination of CEBPA, CEBPB, FOXA1, and FOXA3 was suitable for 201B7 cells to initiate differentiation to the hepatocyte lineage and WE was the most suitable medium for culture after transfection.

F-1066

THERAPEUTIC POTENTIAL OF HUMAN ADIPOSE-DERIVED STROMAL CELLS FOR CRESCENTIC GLOMERULONEPHRITIS IN RAT

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Mesenchymal stromal cells (MSCs) have immunomodulatory function and would be a promising therapeutic agents for inflammatory disorders. Adipose is one of the important source of MSCs, and adipose derived MSCs (ASCs) have some advantages over bone marrow derived cells (BMMSCs) in their proliferative potential and availability. We established our own protocol for ASC culture under low serum conditions (LASCs). When LASCs are compared with ACSs cultured under high serum conditions (HASCs), there are some differences in their secretary capacity of cytokines/ growth factors, immunomodulatory functions, and so on. We have already demonstrated the efficacy of ASCs in some disease models, and LASCs were often more effective than HASCs. In previous series of our experiments, syngeneic ASCs from rat ameliorated crescentic glomerulonephritis (CGN), which causes rapidly progressive glomerulonephritis, and LASCs also showed more efficacy than HASCs and BMMSCs. As a subsequent study, we investigated therapeutic potency and safety of human ASCs for CGN in rat. For inducing CGN, TF78, a monoclonal anti-glomerular basement membrane antibody, was injected to female WKY/NCrj rats. Human LASCs, HASCs or BMMSCs were administrated to them on day 0, 2, 4 each. Therapeutic efficacy was evaluated by proteinuria on day4 and serum creatinine (sCre), BUN, histological renal damage on day 7. As a result, ASCs demonstrated significant therapeutic efficacy compared with BMMSCs. When compared LASC with HASC, HASC appeared to be more effective than LASC although the differences were generally not statistically significant. However, 4 out of 12 rats died in HASC group by unknown cause while there were no deaths in other groups. We think HASCs might have caused fatal pulmonary embolism. This is because many of intravenously administered those MSCs were trapped at lung according to in vivo imaging system, and plasma clotting time of HASC was significantly short in those three kinds of cells. Though ASCs are originally thought to increase the blood's tendency to clot, LASCs seem to be remedied in that point. In this series of experiments, we did show human ASCs have therapeutic potential for CGN as well as ASCs from rat. Given the safety and the efficacy, LASCs might become one of the therapeutic option for CGN.

F-1068

RHO-ASSOCIATED KINASES AND NON-MUSCLE MYOSIN IIS PLAY INHIBITORY ROLES FOR THE DIFFERENTIATION OF HUMAN IPSCS TO PANCREATIC ENDODERM CELLS

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There is increasing success in the generation of pancreatic cells from human pluripotent stem cells, however, the molecular mechanisms of the differentiation remain elusive. The purpose of this study is to address molecular mechanisms for the differentiation towards PDX1+NKX6.1+ pancreatic endoderm cells, which are pancreatic committed progenitor cells. We found that PDX1+ posterior foregut cells differentiated from hiPSCs failed to differentiate into pancreatic endoderm cells at low cell density, but Rho-associated kinase (ROCK) or non-muscle myosin II (NM II) inhibitors rescued the differentiation potential. Although ROCK and its downstream NM II are involved in regulation of cytoskeletal structures, other cytoskeletal modulators, such as inhibitors of actin filament assembly, myosin light chain kinase/phosphatase inhibitors and microtubule inhibitors and stabilizers, failed to induce NKX6.1+ cells. Consistently, the expression of NM IIA and NM IIC was downregulated in aggregation culture which facilitates the induction of PDX1+NKX6.1+ pancreatic endoderm cells (Toyoda T, 2015). NKX6.1+ cells induced by ROCK-NM II inhibitors were observed in a relatively sparse area. In addition, the expression of NKX6.1 was increased by Y-27632 or Blebbistatin treatment under the inhibition of cell proliferation by pre-treatment with a mitotic inactivator, mitomycin C. These findings support the idea that ROCK-NM II inhibition induces pancreatic endoderm cells by mechanisms that mimic effects of aggregation but not by cell proliferation that results in effects of aggregation. The PDX1+NKX6.1+ cells induced with NM II inhibitors were successfully engrafted and maturated in vivo, demonstrating that those are pancreatic endoderm cells that have the developmental potential to differentiate into pancreatic epithelia. Taken together, these results suggest that NM IIs play inhibitory roles for the differentiation of hiPSCs to pancreatic endoderm cells. Our findings may provide molecular basis of the development of hESC/iPSC-based cell source.

F-1070

DEVELOPMENTAL ORIGINS OF DISEASE: INSIGHTS FROM FETAL ALCOHOL SPECTRUM DISORDER

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The developmental origins of adult disease (DOAD) model postulates that fetal and perinatal events can impact the development of organ progenitors and function to significantly contribute to adult health outcomes. One common developmental stressor is fetal alcohol exposure. Alcohol and its metabolite acetaldehyde are teratogens, and alcohol consumption during pregnancy can negatively impact the developing fetus and lead to fetal alcohol spectrum disorders (FASDs). The teratogenic effects of alcohol are thought to have lifelong consequences; however, the impact of fetal alcohol exposure on adult health outcomes is poorly characterized. In order to examine the impact of fetal alcohol exposure on adult disease phenotypes, we assessed body mass index (BMI) and blood glucose homeostasis, with the ultimate goal of gaining insights into the molecular and metabolic mechanisms responsible. We have successfully induced developmental features of FASDs in zebrafish by embryonic alcohol exposure, including craniofacial and renal developmental abnormalities. Fetal alcohol exposed (FAE) embryos were raised to adulthood and challenged with a high fat/high cholesterol diet FAE adult zebrafish. FAE males undergo significant diet-induced BMI gains while controls without fetal alcohol exposure do not. Food consumption assays reveal no alterations in eating behavior, indicating the possibility that developmentally-acquired defects are responsible for the increased adiposity observed. Our studies provide proof-of-concept that in the presence of a dietary environmental stressor, fetal alcohol exposure can be a risk factor for BMI gain and obesity in males. Our ongoing studies probe the impact of FAE on metabolism, fatty liver incidence, and altered blood glucose homeostasis.

Funding Source: Sternlicht Director's Fund Fellowship, Harvard Stem Cell Institute

F-1072

DEVELOPMENT OF AN IN VITRO HUMAN LIVER MODEL BY IPSC-DERIVED PARENCHYMAL AND NON-PARENCHYMAL CELLS

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Hepatocytes, liver parenchymal cells, play a central role for drug metabolism by expressing a number of cytochrome P450 oxidases, and are important for toxicology and drug development. As primary hepatocytes isolated from livers lose their functions quickly and their supply is limited, there are many studies reporting hepatic differentiation of human induced pluripotent stem cells (hiPSCs). However, the levels of mature hepatic functions, in many cases, are not high enough as compared with primary human hepatocytes. Because hepatoblasts proliferate and differentiate into mature hepatocytes through interactions with hepatic non-parenchymal cells (NPCs) such as liver sinusoidal endothelial cells (LSECs) and hepatic stellate cells (HSCs), we reasoned that it is necessary to reproduce liver development in vitro to make mature hepatocytes. In order to make a functional liver model in vitro, we previously established a system to generate liver progenitor cells (LPCs) from hiPSCs. In this study, we generated LSECs and HSCs from hiPSCs. We found that TGFβ and Rho signaling pathways, respectively, regulate the proliferation and maturation of LSECs and HSCs isolated from mouse fetal livers. Based on these observations in mice, we established the differentiation protocols to derive LSECs and HSCs from hiPSCs. These hiPSC-derived LSECs and HSCs exhibited characteristics similar to each cell type. Co-culture of LPCs with NPCs from hiPSCs allowed LPCs to proliferate and differentiate to express various hepatocyte enzymes at levels much higher than the hepatocytes derived from iPSCs without NPCs. Thus, hiPSC-derived NPCs are useful for generating functional liver tissue in vitro and the hiPSC-derived liver model will be useful for drug screening, pathological models, and cell therapy.

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EPITHELIAL TISSUES

F-1076

MICRORNA PROFILING OF ENRICHED HUMAN CORNEAL EPITHELIAL STEM CELLS

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The corneal epithelium covers the anterior surface of the eye and its homeostasis is maintained by the corneal epithelial stem cells (CESCs) residing in the basal layer of limbus at the corneo-scleral junction. These stem cells constitute 3-5% of the total limbal epithelium. Since there is no exclusive marker for their isolation, understanding the molecular mechanisms regulating the maintenance of stemness is still not clear. In this study a 80% enrichment of CESCs was obtained by i) differential enzymatic treatment to isolate the basal limbal epithelial cells followed by ii) laser capture micro dissection of cells with nucleus to cytoplasm ratio ≥ 0.7, using donor tissues obtained from Rotary Aravind International Eye Bank, Madurai. Total RNA was extracted from enriched CESCs and differentiated central corneal epithelial cells (CCECs) and microRNA (miRNA) expression profiling was carried out using Illumina Nextseq 500 platform. Small RNA sequence data were aligned to Homo sapiens hg19 genome reference, allowing for one mismatch using bowtie1 aligner in the sRNAbench tool and mature miRNAs were annotated using miRBase (release 21). A total of 62 miRNAs were identified in CESCs and 611 miRNAs in CCECs. R tools were used to identify significantly differentially expressed miRNAs in CESCs. The top ten miRNAs upregulated in CESCs included, (i) hsa-miR-21-5p, hsa-miR-3168, hsa-miR-143-3p and hsa-miR-99b-5p with ≥10 fold change, (ii) stem cell proliferation and maintenance specific hsa-miR-21-5p, hsa-miR-191-5p, hsa-miR-26a-5p, hsa-miR-10a-5p, hsa-miR-99b-5p and (iii) novel, embryonic stem cell specific hsa-miR-3168 and hsa-miR-1910-5p. Target prediction of these ten miRNAs identified 2181 targets that were further annotated by DAVID. 33 KEGG pathways were significantly regulated ($p < 0.05$;FDR < 0.01), including signaling pathways that regulate pluripotency of stem cells, focal adhesions, toll like receptors, PI3-AKT pathway, MAPK pathway, cancer related pathways and neurotrophin signaling pathways that regulate cell migration, growth and proliferation. Of these, the 54 genes involved in signaling pathways regulating pluripotency of stem cells is of interest to explore further.

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F-1078

TISSUE-SCALE COORDINATION OF CELLULAR BEHAVIOR PROMOTES EPIDERMAL WOUND REPAIR IN LIVE MICE

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Tissue repair is fundamental to our survival as tissues are challenged by recurrent damage. During mammalian skin repair, cells respond by migrating and proliferating to close the wound. However, the coordination of cellular repair behaviors and their effects on homeostatic functions in a live mammal remains unclear. Here we capture the spatiotemporal dynamics of individual epithelial behaviors by imaging wound re-epithelialization in live mice. Differentiated cells migrate while the rate of differentiation changes depending on local rate of migration and tissue architecture. Cells depart from a highly proliferative zone by directionally dividing towards the wound while collectively migrating. This regional co-existence of proliferation and migration leads to local expansion and elongation of the repairing epithelium. Finally, proliferation functions to pattern and restrict the recruitment of undamaged cells. This study elucidates the interplay of cellular repair behaviors and consequent changes in homeostatic behaviors that support tissue-scale organization of wound re-epithelialization.

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F-1080

BNIP3-INDUCED AUTOPHAGY IS REQUIRED FOR MAINTENANCE OF EPIDERMAL HOMEOSTASIS

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The skin epidermis is a stratified epithelium that plays an important role in a barrier function. Previously, we found that Hes1 was required for maintenance of the immature status of suprabasal cells by preventing premature differentiation. Transcriptome analysis of *Hes1*$^{-/-}$ mouse revealed the direct relationship between Hes1 and BNIP3, a potent inducer of autophagy. Keratinocyte differentiation is going along with activation of lysosomal enzymes and organelle clearance, expecting the contribution of autophagy in this process. In this study, we found that BNIP3 was expressed in the suprabasal layer of the epidermis, where autophagosome formation is normally observed. Forced expression of BNIP3 in human primary epidermal keratinocytes (HPEKs) resulted in autophagy induction and keratinocyte differentiation, whereas knockdown of BNIP3 had the opposite effect. Intriguingly, addition of an autophagy inhibitor significantly suppressed the BNIP3-stimulated differentiation of keratinocytes, suggesting that BNIP3 plays a crucial role in keratinocyte differentiation by inducing autophagy. Furthermore, our data suggests that BNIP3 is also important for maintenance of skin epidermis. Intriguingly, although UVB irradiation stimulated BNIP3 expression and cleavage of caspase3, suppression of UVB-induced BNIP3 expression led to further increase in cleaved caspase3 levels. This suggests that BNIP3 has a protective effect against UVB-induced apoptosis in keratinocytes. Furthermore, we found that the accumulation of reactive oxygen species (ROS) by UVB irradiation was sufficient to trigger the activation of JNK and ERK mitogen-activated protein kinase (MAPK) in HPEKs. In turn, activated MAPK mediated the upregulation of BNIP3 expression. Treatment with an anti-oxidant reagent or a specific inhibitor of MAPK significantly attenuated the expression of BNIP3 triggered by UVB, followed by the induction of cell death by apoptosis. Furthermore, UVB-induced apoptosis was significantly stimulated by chloroquine or bafilomycin A1, an inhibitor of autophagy. Moreover, BNIP3 was required for the degradation of dysfunctional mitochondria upon UVB irradiation. Overall, our data provide valuable insights into the role of BNIP3 in the maintenance of epidermal homeostasis.

F-1082

AN IN VITRO CORNEAL EPITHELIAL STEM CELL NICHE MODEL WITH LONG-TERM HOMEOSTASIS AND WOUND HEALING CAPABILITY

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We have previously reported a protocol to maintain primary limbal epithelial sheets for over 6 months. We further sought to determine if epithelial cells cultured as a stratified layer with proper cell polarity can maintain homeostasis in vitro for over one year and maintain homeostasis and wound healing. Human corneal limbal epithelial cells including progenitor cells were maintained in DMEM/F12 based medium containing fetal bovine serum (4%), human FGF7, Y27632, insulin, hydrocortisone, tri-iodo-thyronine and isoproterenol. Immunohistochemistry was done for epithelial markers, and cell proliferation following wound-healing assays were observed by Fluorescent ubiquitination-based cell cycle indicator (Fucci) and EdU pulse labeling. Human limbal epithelial cells spontaneously formed a polarized, stratified epithelial sheet. Cell sheets continued to shed cells daily for 1 year. Cell turnover rate and the expression pattern of the stem cell marker, KRT15 reached a steady state within 3 months, at which point densely packed clusters of KRT15 positive cells associated with dendritic melanocytes became apparent. Fluorescent ubiquitination-based cell cycle indicator (Fucci) and EdU pulse labeling showed that cells initially migrated into the wound, followed by robust proliferation. Fluorescence was easily observed in live cells due to the transparent nature of the cell sheet. This novel epithelial layer culture technique will be a powerful tool in the study of human epithelial stem cell homeostasis and wound healing.

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F-1084

ORDERED ARCHITECTURE OF SKIN HAIR FOLLICLE EPITHELIUM IS SUSTAINED BY A DYNAMIC CELLULAR FLUX

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Tissue architecture is crucial for tissue function. Yet we still don't understand the cellular and signaling mechanisms that stem cells and their differentiated progeny adopt to establish a proper tissue architecture.

The hair follicle provides a unique model system to interrogate such mechanisms because it periodically rebuilds its architecture during stereotypic hair cycles. Previous work showed that hair progenitors are spatially organized at the bottom of the follicle onto a dome-like cellular platform from which the several differentiated cell layers are generated and centrically converge to form the hair shaft. How this ordered architecture is built from a handful of stem cells (SCs) remains unclear. By tracking the same cells in the same live mice over time, we show that SCs are amplified and initially organized in a stereotypic manner. Specifically, the position of each SC at the onset of hair follicle growth restricts the fate to a specific group of hair progenitors. Unexpectedly, as the progenitor platform is established at the bottom of the follicles, the hair progenitors do not appear to be restricted to specific fates any longer but rather directionally and dynamically change their position suggesting flexibility in cell fate choices. This cellular flux appear to follow a gradient of Wnt activation which is continuously active as shown by FRAP analysis of a live Wnt reporter. Current gain- and loss-of-function single cell manipulation studies are addressing the causality of Wnt relationship to the observed flexibility in hair progenitor cell fate choices. This study underscores the dynamic cellular and signaling mechanisms employed to establish a highly ordered tissue architecture.

F-1086

RUNX1 REMDODELS THE CELLULAR LIPID METABOLISM OF HAIR FOLLICLE STEM CELLS

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The role of lipid metabolism in tissue homeostasis and stem cell function is poorly understood. Previously our laboratory uncovered the hematopoietic SC transcription factor Runx1 as an important HFSC regulator during adult skin homeostasis in mouse. We found that Runx1 was important for adult HFSC timely activation from quiescence and for growth a HFSC in culture (1). Our data suggest that Runx1 expression in these quiescent bulge SCs promotes an early progenitor (hair germ) cell-state, which is prone for subsequent rapid activation and proliferation (1). Moreover, we found Runx1 was required for mouse and human skin and oral epithelial carcinogenesis by promoting proliferation (2). To understand how Runx1-driven gene expression changes may contribute to proliferation, we focused on a group of genes related to lipid metabolism that were over-represented in a microarray data set from sorted HFSCs with elevated levels of Runx1. Specifically, we focused on the Scd1 and Soat1 enzymes, which were previously reported to promote proliferation in other systems. Lipidome analysis revealed changes in the concentration of mono-unsaturated fatty acids, which are reaction products of Scd1 in Runx1 mutant skin. Further probing our lipidome data to cellular attributes, we found fluidity of the plasma membrane, which is influenced by the composition in unsaturated fatty acids, to be altered with varying expression of RUNX1. We also found by inhibition experiments that SCD1 and SOAT1 are synergistically necessary for the proliferation of mouse keratinocytes and human squamous cell carcinoma lines from skin and the oral epithelium. Our results suggest that Runx1 remodels the cellular lipid metabolism and the plasma membrane properties by controlling the expression of specific lipid-related enzymes, thus providing the required conditions for rapid activation and cell growth of normal HFSCs and of epithelial skin cancer cells.

STEM CELL NICHES

F-1088

OPTOGENETIC INVESTIGATION OF EFFECTS OF BETA-CATENIN SIGNALING DYNAMICS ON ADULT NEURAL STEM CELL DIFFERENTIATION

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Adult neural stem cells (NSC) generate neurons in specific regions of the brain throughout adulthood, play key roles in memory and neurological disease, and offer attractive therapeutic targets. The microniches for NSCs are likely highly dynamic and present signaling molecules that fluctuate on time scales ranging from neurotransmitter signaling to long term behavioral changes. Adult hippocampal NSC exposure to Wnt ligands activates β-catenin to induce neuronal differentiation in vivo and in vitro. To investigate the impact of Wnt fluctuations, as a model for niche signaling dynamics on NSC function, our lab developed a tunable optogenetic system to modulate β-catenin signaling via Cry2 oligomerization of the LRP6 intracellular domain. Similar to Wnt3a addition, illumination of NSCs expressing Cry2-LRP6c induced neuronal differentiation. This outcome enables several questions: do NSCs differentiate when the integral of signaling during a temporal window exceeds a key threshold, or do dynamics in signal presentation matter? Continuous illumination at different light intensities in vitro resulted in a saturable dose response. However, exposure to alternating TON/TOFF illumination at varying frequencies, to simulate signaling dynamics over a range of times scales, yielded different results. Low frequency illumination yielded considerably less neuronal differentiation than in cells

that received the same overall signal dosage but with continuous illumination. Furthermore, low frequency illumination increased apoptosis, indicating exposure to this signal rendered cells dependent upon it not only for differentiation but also survival. Whole transcriptome sequencing revealed candidate genes implicated in differentiation and survival. Overexpression of one cell cycle regulatory factor, whose endogenous expression is lost upon Wnt signal withdrawal, significantly decreased apoptosis and rescued differentiation upon signal loss. These results indicate potentially critical survival roles for cell cycle exit and prevention of aberrant cell cycle re-entry upon loss of β-catenin activity. In sum, we harness optogenetics to demonstrate temporal dynamics in signaling presentation can exert a strong impact on stem cell behavior, offering further insights into the biology of adult neurogenesis.

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F-1090

E3 UBIQUITIN LIGASE MULE TARGETS B-CATENIN UNDER CONDITIONS OF HYPERACTIVE WNT SIGNALING

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Wnt signaling plays critical roles both in embryonic development and the maintenance of homeostasis in many adult tissues. Two particularly important cellular programs orchestrated by Wnt signaling are proliferation and stem cell self-renewal. Constitutive activation of the Wnt pathway due to mutation or improper modulation of pathway components contributes to cancer development in various tissues. Colon cancers frequently bear inactivating mutations of the adenomatous polyposis coli (APC) gene, whose product is an important component of the destruction complex that regulates b-catenin levels. Stabilization and nuclear localization of b-catenin result in the expression of a panel of Wnt target genes. We previously showed that Mule/ Huwe1/Arf-BP1 (Mule) controls murine intestinal stem and progenitor cell proliferation by modulating the Wnt pathway via c-Myc. We now extend our investigation of Mule's influence on oncogenesis by showing that Mule interacts directly with b-catenin and targets it for degradation under conditions of hyperactive Wnt signaling. Our data shows that the combined loss of Mule and APC accelerates the conversion of normal intestinal stem cells into cancer stem cells, paving the way to colorectal cancer development. Over the past year, we and others have exploited different Mule knockout mouse models to demonstrate that Mule is involved in the regulation of intestinal, hematopoietic and neural stem cells. Significantly, the developmental programs of all these tissues are driven by the Wnt pathway. In the absence of Wnt, the protein stability of b-catenin is strictly controlled by the destruction complex, resulting in its phosphorylation and subsequent ubiquitination by b-TRCP. Our study shows that at least in a hyperproliferative setting, Mule can serve as a "back up" E3 ubiquitin ligase to b-catenin to target it for degradation and thereby help to quench Wnt signaling in the intestine. It remains to be determined whether this regulation is tissue-specific or extends to other Wnt-driven tissues. Nevertheless, our findings suggest that Mule utilizes various mechanisms to fine-tune the Wnt pathway and provides multiple safeguards against tumorigenesis.

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F-1092

LOSS OF JEDI-1 RESULTS IN INCREASED PROLIFERATION OF NEURAL PROGENITOR CELLS

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We previously identified Jedi-1 as an engulfment receptor necessary for clearance of apoptotic sensory neurons in the dorsal root ganglia during normal development (Wu et al, 2009). Its role in the central nervous system, however, has not been investigated. In the perinatal (postnatal day 6) mouse brain, we detected Jedi-1 expression primarily in endothelial cells, using a LacZ reporter. However, we also found Jedi-1 mRNA expressed in neural progenitor cells (NPCs) derived from the ventricular-subventricular zone (V-SVZ), one of the two niches where neurogenesis continues during adult life. Interestingly, NPCs derived from perinatal jedi-1-/- mice exhibited a significant increase in proliferation relative to wild type ($p=0.002$), as measured by short term BrdU incorporation. Most proliferating cells were GFAP+ and Nestin+, common markers of early NPCs. As previously reported (Lu et al, 2011), we found that NPCs have the ability to engulf carboxylated microspheres. However, preliminary data indicate that jedi-1-/- NPCs

do not have a deficit in engulfment capability, suggesting that Jedi-1 regulates proliferation of these cells through an alternate mechanism. Current experiments are focused on determining the mechanistic basis for Jedi-1's effects on proliferation in vitro and in vivo.

F-1094

HIERARCHY OF THE EMT GRADIENT IN MESENCHYMAL NICHE REPRESENTS A NICHE ACTIVATION PROGRAM IN RESPONSE TO REGENERATIVE MICROENVIRONMENT

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Mesenchymal niche in bone marrow (BM) comprise a key cellular element of microenvironment to regulate hematopoietic stem cells (HSCs) behavior. While studies have identified specific subsets of mesenchymal cells as niche, the characteristic signatures for their niche activity remains unknown. Here, we show that mesenchymal cells acquire enhanced niche activity for normal and cancer stem cells when engaged into 3-dimensional cell interaction, and that this enhancement is associated with further progression of epithelial-mesenchymal transition (EMT) and creation of EMT gradient among mesenchymal cells, driven by shift of EMT-related miRNAs/transcription factors. The enhanced niche activity by EMT was similarly reproduced in 2D adherent mesenchymal cells by EMT-promoting miRNAs, whereas knock-down of EMT factors caused decrease of niche activity. Moreover, increase of EMT gradient in niche activation was similarly observed in the physiological stimuli inducing self-renewal/proliferation of HSCs, such as poly (I:C), substance P, or ex-vivo culture conditions supporting higher self-renewal of HSCs. Interestingly, throughout the models for niche activation, increase of EMT gradient was bound to the acquisition of stem cell-like chromatins and niche markers, indicating a conserved 'niche activation program' for niche activation. Furthermore, transcriptomic analysis of nestin-GFP knock-in mice model showed that subsets of mesenchymal cells serving as in-vivo niche (nestin+PDGFR+) exhibited higher EMT gradient towards more naïve mesenchymal status than other cells deficient in niche activity. In contrast, mesenchymal cells exposed to leukemic blast of AML exhibited rather decreased shift of EMT gradient compared to cells exposed to normal HSCs, indicating their responsive nature to physiological microenvironment. Thus, the molecular hierarchy in the EMT gradient among phenotypically redundant mesenchymal cells revealed previously unrecognized functional parameters for mesenchymal niche activity, representing it as a reactive stromal response to physiological stimuli from microenvironment. Our study should also shed light on the paracrine niche-based mesenchymal cell therapeutic trials for more predictable outcomes in therapeutic efficacy.

F-1096

CYTOTOXIC THERAPIES PERMANENTLY ALTER THE MICROENVIRONMENTAL SUPPORT OF MURINE MULTIPOTENT PROGENITOR CELLS AND HEMATOPOIETIC STEM CELLS

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Cytotoxic therapies used for the treatment of cancer are known to dramatically affect hematopoiesis, however, the response of different niche cell types that regulate hematopoietic stem cells (HSCs) and progenitor cells to cancer therapies are largely unknown. Using adult male mice, we have extensively analysed the effects of irradiation and a bone marrow transplant or a single dose of the chemotherapy agent, 5-fluorouracil (5-FU), on different niche cells at early and late time points post-therapy. We have also investigated changes in HSCs and progenitor cells at all time points. All therapies caused early, transient changes in various niche cell types. Irreversible loss of bone occurred in response to each therapy and was also observed in allogeneic transplant patients. Therapy-specific changes were also observed for other niche cell types (adipocytes and vasculature). The numbers of multipotent progenitor cells (MPPs) were significantly reduced post-therapy and correlated with significantly impaired repopulation capacity of the bone marrow cells obtained post-therapy. In contrast, HSCs obtained post-therapy had similar competitive repopulating capacity compared to HSCs obtained from age-matched controls. Studies using inducible HSC-specific Confetti reporter mice pulsed for a short time to clonally track HSCs and MPPs showed that there were significant increases in the numbers of Confetti positive HSCs detected in mice post-cancer therapy. However, the numbers of Confetti positive MPPs produced per Confetti positive HSC were significantly reduced. Additional data using membrane-targeted tandem dimer tomato/membrane-targeted GFP (mTmG) Flk2Cre reporter mice and preliminary molecular studies have suggested that there is a reduced ability of ST-HSCs to generate sufficient numbers of

MPPs post-cancer therapies. Our findings suggest that the production of MPPs from ST-HSCs is significantly impaired long-term post-cancer therapies, likely due to impaired extrinsic (niche) regulation. Hence the identification and characterization of the niche where ST-HSCs transition to MPPs may lead to treatments aiding hematological recovery after cancer therapies.

EYE AND RETINA

F-1100

TOWARDS A CELL THERAPY FOR GLAUCOMA – TRANSPLANTATION OF MOUSE IPSC-DERIVED RETINAL GANGLION CELLS

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The loss of retinal ganglion cells (RGCs) within the retina remains one of the major causes of vision loss in the developed world, with Glaucoma being proposed as the second leading cause of blindness worldwide. To date, available pharmacological interventions are unable to halt the associated progressive loss of RGCs. Following recent advances in stem cell research and cell therapeutics, transplantation of donor derived RGCs as well as ES-derived retinal progenitors led to successful survival and integration of RGC(-like) neurons into the retina, suggesting the potential to achieve both, neuroprotection and cell replacement by RGC transplantation. Nevertheless, for clinical translation a better source of RGCs, allowing for selective isolation of fully differentiated RGCs at high efficiency, will be required to improve transplantation outcome and reduce tumor risk. We here document the generation/isolation of in-vitro differentiated RGCs from a mouse-derived Thy1-GFP iPS cell line in which RGC differentiation was induced by employing a 3D cell culture approach, leading to the generation of optic cups over the course of 21 days. After optic cup maturation, RGCs were isolated by magnetic micro-beads targeting Thy1, a surface glycoprotein uniquely expressed in RGCs allowing for their selective isolation from the derived optic cups. The efficiency of differentiation was about 10% with selected cells expressing markers for RGC: Brn3a, Islet1, Thy1 and Math5. While previous data from our laboratory already demonstrated survival of those Thy1+ RGCs after syngeneic transplantation into donor retinas, it remained unclear whether they can functionally integrate in the recipient retina. To address that question isolated Thy1+ RGCs were intravitreally injected into p2 to p4 aged mouse pups (10,000 cells in 1µl), utilizing the permissive environment of a developing retina. Three weeks post-injection both in-vivo imaging and subsequent immunohistochemistry prove integration of donor cells as well as elaborate axonal outgrowth. In addition, synaptic connections between the injected RGCs and the host retina were found. iPSC are a reliable source for differentiated RGCs for transplantation, with their rate of integration warranting further study in animal models of glaucoma and optic neuropathies.

F-1102

IDENTIFICATION AND QUANTIFICATION OF HUMAN TRABECULAR MESHWORK STEM CELLS

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The presence of stem-like cells in trabecular meshwork (TM) was first identified based on active cell proliferation in the anterior non-filtering part of TM (Schwalbe's line/insert region) after laser trabeculoplasty in human corneoscleral explant organ cultures. Recent reports in primate and non-primate eyes have reported the presence of BrdU label retaining cells in the insert region. This study aims to locate the stem cells in human TM and to quantify their content using donor tissues obtained from Rotary Aravind International Eye Bank, Madurai. The donor tissues were used either for cryosectioning (n=3) or for native TM cell isolation by collagenase A treatment (n=5). The cryosectioned donor tissues/ cytosmears of isolated native TM cells were immunostained for ATP Binding Cassette protein G2 (ABCG2) and analysed using Leica SP8 confocal microscope. ABCG2 expression was higher in the Schwalbe's line region and the Juxtacanalicular (JCT) region compared to other parts of TM. The ABCG2 immunostained cells were analysed for two parameters - the mean pixel intensity of the membrane staining (pixel intensity of 177±38 was graded as high expression, 75±20 as low expression and 34±6 as negative) and nuclear to cytoplasmic (N/C) ratio. This method was established earlier in our laboratory as a specific method to quantify corneal epithelial stem cells. Similar analysis using TM cells identified 11±1 % of TM cells to have a high expression of ABCG2 with high N/C ratio. These results confirm the presence of stem-like cells in human TM, more specifically in the insert region and JCT. Further studies are being carried out using additional stem cell markers and functional analysis to confirm their adult stem cell property. Thus, establishing a method for the identification and quantification of human TM stem cells will be of significance to understand their role in the maintenance of tissue homeostasis.

Funding Source: Aravind Eye Hospital, Madurai

F-1104

THE DEVELOPMENTAL STAGE OF HUMAN RETINAL PIGMENT EPITHELIUM CELL INFLUENCES TRANSPLANT EFFICACY FOR VISION RESCUE

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Age-related macular degeneration (AMD) is the leading cause of vision loss in the elderly. AMD is accompanied by degeneration of Retinal pigment epithelium (RPE) cells, a monolayer of cells involved in supporting the overlying photoreceptor cells. RPE transplantation therapies hold promise to slow the disease progression. The use of embryonic stem cells derived RPE and induced pluripotent stem cell derived RPE is being explored for transplantation, and critical concerns regarding mis-differentiation and tumorigenicity arising from residual pluripotent stem cells are being addressed. Previously, we identified the existence of a rare (less than 10%) subpopulation of RPE stem cells (RPESC) extracted from the adult human RPE layer (Salero, 2012). RPESC can self-renew extensively and are poised to generate highly pure RPE cultures that express key features of native RPE. We report the successful production of GMP banks of RPESC from three different donors. Sub-retinal transplantation of a suspension of RPESC-derived RPE rescues visual function in Royal college of Surgeons (RCS) rats. Remarkably, the stage of differentiation that RPESC derived RPE acquire prior to transplantation influences the efficacy of vision rescue. We tested three stages of RPE cell maturation - 2 weeks, 4 weeks and 8 weeks. Whereas cells tested at all stages of differentiation rescue photoreceptor layer morphology, an intermediate stage of RPESC derived RPE differentiation obtained after around 4 weeks of culture was more consistent at vision rescue than progeny that were differentiated for 2 weeks or 8 weeks. We observed 95% retention of normal visual function in the RCS rats after transplantation of 4 week cells, which was significantly more than the 2 week and 8 week cells. Our results indicate that the developmental stage of adult RPESC derived RPE cells influences the efficacy of RPE cell replacement therapy.

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F-1106

HUMAN PLURIPOTENT STEM CELLS MODELING BIETT'S CRYSTALLINE RETINAL DYSTROPHY BY USING CRISPR/CAS9 TECHNOLOGIES

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Bietti's crystalline retinal dystrophy (BCD) is an inherited disease caused by mutations of CYP4V2 gene (an enzyme involved in a multi-step process of fatty acid oxidation). It is characterized by numerous tiny crystal lipids deposits at the cornea and retinal pigmented epithelial cells (RPE), which ultimately lead to retinal degeneration. The transgenic rodent model of BCD failed to truly recapitulate human BCD. Because the pathophysiology of BCD-caused RPE damage is poorly understood, the BCD is incurable and there is a compelling need for new therapies to rescue RPE cells death caused by BCD and further improve clinical outcome. By integration of CRISPR/cas9 technology and pluripotent stem cells , it enables us possible to understand human disease pathophysiology that is unavailable in animal models. Three patient-specific and healthy donors' induced pluripotent stem cells (iPSC) have been generated using the peripheral blood mono nuclear cells (PBMC) . All the iPS cells are integration-free and have been well characterized for RPE differentiation and gene correction. We also used CRISPR/cas9 technology to mutate CYP4V2 in healthy iPSC, and correct patients' gene mutation in iPS cells. The profile of fatty acid metabolites in stem cell and RPE cells are analyzed. Some different fatty acid metabolites have been identified. The results of this study should provide important insight into the development of gene correction-based autologous RPE cell therapy to rescue genetic RPE defects and improve retinal function in BCD.

NEURAL DEVELOPMENT AND REGENERATION

F-1108

TOWARD HAIR CELL REGENERATION: HIC1 REGULATION OF ATOH1

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Mammalian hair cells, the sensory cells that respond to sound, do not regenerate to a significant degree once lost, and their loss is a major cause of deafness. Progenitor cells in the cochlea, marked by expression of leucine-rich repeat containing, G-protein-coupled receptor 5 (Lgr5), have the capacity for self-renewal and hair cell differentiation. Formation of hair cells relies on the expression of Atonal 1 (Atoh1), a key regulator of hair cell fate. Among the transcriptional regulators of the Atoh1 gene, Hypermethylated in Cancer 1 (Hic1) has been shown to inhibit Atoh1 in the cerebellum and its expression increases with age. Here we show that Hic1 inhibits Atoh1 through its interaction with the Atoh1 DNA regulatory enhancer directly as well as by sequestering β -catenin and preventing its activity on the enhancer. Using primary inner ear organoid culture system, inhibition of Hic1 drives Atoh1 expression and generation of hair-cell like cells from undifferentiated progenitor cells. Finally, we show change in expression of Hic1 in the mammalian (mouse) cochlea with age. Our findings suggest that Hic1 contributes to the silencing of Atoh1 within the cochlea and may represent an important target in our in vivo regeneration efforts.

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F-1110

IDENTIFICATION OF DRUGS ACTING SPECIFICALLY ON NEURAL STEM/ PROGENITOR CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS BY USING A TIME-COURSE CYTOTOXICITY TEST

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Since the development of human induced pluripotent stem cells (hiPSCs), various cell types have been established for regenerative medicine and drug development, preventing ethical issues or technical difficulties regarding obtaining human tissue-derived cells. hiPSC-derived neural stem/progenitor cells (hiPSC-NSPCs) were shown to have beneficial effects in the treatment of spinal cord injury and stroke. However, intrinsic proliferative potential of transplanted cells presents the risk of undesired growth before terminal differentiation in specific microenvironments in vivo, although such proliferation did not result in teratoma formation or carcinogenesis. Recently, pretreatment of hiPSC-NSPCs with a γ-secretase inhibitor before transplantation has been reported to prevent overgrowth in vivo. However, targeted cytotoxic drugs specifically acting on transplanted hiPSC-NSPCs are still needed after transplantation as a safety net for stem cell-based therapies. In this study, we examined the effects of approved anticancer drugs cisplatin, etoposide, mercaptopurine, and methotrexate on hiPSC-NSPCs and human neural tissue-derived NSPCs (hN-NSPCs) by performing a 7-day time-course assay and a conventional ATP assay on day 2. We classified four anticancer drugs as fast- or slow-acting using a time-course cytotoxicity test, and showed that slow-acting drugs distinguish hiPSC-NSPCs from hN-NSPCs, which was not evident in the conventional ATP assay performed on day 2. Because hN-NSPCs have higher tolerance for slow-acting drugs compared to hiPSC-NSPCs, we propose that slow-acting drugs, such as methotrexate, can be candidates for the safety net of hiPSC-NSPCs-based therapies, preventing undesirable cell proliferation after transplantation.

F-1112

TRANSPLANTATION OF MOUSE ES CELL DERIVED NEURAL PROGENITORS IMPROVES HEARING IN A MOUSE MODEL OF AUDITORY NEUROPATHY

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Sensorineural hearing loss (SNHL) is a widespread sensory problem and is increasing in prevalence. Degeneration of auditory neurons is one of the major causes. Auditory neurons do not regenerate after damage and cellular therapies, such as transplantation of stem cell derived neural progenitors area promising therapeutic approach for the restoration of neural circuits. Mouse ES cells were subjected to a neural induction protocol to derive neural progenitors that expressed peripheral sensory neuron markers. The auditory nerve trunk was accessed via a standardized stereotaxic approach in our previously established auditory neuropathy mouse model, in which auditory neurons are pharmacologically ablated. Progenitors were transplanted into the auditory nerve

trunk one week after damage. Successful engraftment was confirmed by immunohistochemistry 10 days and 4 weeks after transplantation. Functional response was measured by auditory brainstem evoked responses (ABRs) before and after auditory nerve ablation, and 4 weeks after transplantation of the cells. We demonstrate that sensory neural progenitors can be derived from mouse ES cells and can be successfully integrated into the peripheral auditory circuit with improvement of hearing loss after nerve damage. This proof of concept study represents a first step toward future cell based regeneration strategies for hearing loss.

F-1114

DETERMINING THE ROLE OF PRIMITIVE AND DEFINITIVE NEURAL STEM CELLS IN A NEONATAL MOUSE MODEL OF HYPOXIA-ISCHEMIA BRAIN INJURY

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Perinatal hypoxia can result in long-term neurological deficits, including cerebral palsy (CP). Our lab has successfully activated resident neural stem cells and their progeny (together termed neural precursor cells, NPCs) using metformin, which promotes neurogenesis, oligogenesis, and correlates with functional recovery following hypoxia ischemia (HI). Herein, we sought to determine if subependyma-derived NPC activation is necessary and/or sufficient to promote functional recovery following HI. We used a GFAP-tk mouse model to selectively ablate NPCs with delivery of ganciclovir (GCV) and 1-β-arabinofuranosylcytosine (Ara-C) in strategies that target definitive neural stem cells only (dNSCs) (GCV) or dNSCs and progeny (GCV and Ara-C). We established early (P4-7) and late (P11-14) ablation paradigms to investigate changes in the cell profile before and after HI (P8). We achieved ~90% ablation of NPCs using both strategies in the postnatal brain and monitored repopulation with the neurosphere assay. As predicted, 48% repopulation is observed two weeks following GCV alone, but the 90% loss is maintained for up to 2 months after GCV+Ara-C. Since dNSCs did not return following GCV and Ara-C treatment, we investigated changes in the upstream primitive neural stem cell (pNSC) population. With GCV and Ara-C, pNSC decline (40% to 50% in early and late ablation, respectively) but recover in 2 weeks. Surprisingly, we saw 30% fewer pNSCs following GCV treatment alone after early ablation. We propose that transient GFAP expression occurs in early post-natal pNSCs – something not observed in the adult lineage and are examining this with FACS and RNA-seq. Importantly, we are investigating the effects of NPC ablation on functional recovery following HI+met administration. When we ablate cells prior to HI, we observe a return of NPCs to 30% of control levels at 4 days post-HI. We are optimizing our ablation strategy to maintain the loss of NPCs, since ablation offers an ideal paradigm to study their role in injury and repair. These analyses will provide insight to the cell based mechanisms that underlie functional recovery observed with endogenous activation strategies.

F-1116

CONTEXT-DEPENDENT HOXB1 FUNCTION AND HARNESSING ITS POTENTIAL TO GENERATE FACIAL MOTOR NEURON PROGENITORS

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The genetic mechanisms underlying the developmental and functional specification of the multitude of neural cell types remain the focus of intense interest. Hox genes play important roles in development by regulating the specification of positional identities along the anterior-posterior axis of the developing embryo. Hoxb1 in particular plays a vital role in the specification of facial branchiomotorneurons (FBM) that innervate facial muscles and it is also strongly expressed during early development in the most posterior part of the embryo in the dual–fated neuromesodermal progenitors (NMPs). To investigate the mechanisms of Hoxb1 action in these different developmental contexts we are using directed differentiation of mES cells into FBM and NMPs. Expression profile analysis of these progenitors revealed several distinct candidate Hoxb1 target genes. In mES derived FBM progenitors, Hoxb1 induces expression of the receptor tyrosine-protein kinase RET. This was confirmed by analyzing RET expression in wild type and Hoxb1 null mouse embryos. In NMPs, Hoxb1 promotes cell survival by regulating expression of Fgf8 and the proapoptotic gene Trib3. To confirm that these are direct Hoxb1 targets and identify additional context-dependent Hoxb1 targets we are carrying out ChiP-Seq analyses using mES derived FBM progenitors and NMPs. There are many devastating diseases affecting motor

neurons. The ability to generate motor neurons of distinct identity in vitro would be of great value in disease modeling and possibly cell therapy. We are assessing the suitability of our mES derived FBM progenitors by orthotopically grafting into the facial nucleus of adult wild type mouse. The transplanted cells survive for at least six months and extend axons indicating that they may undergo maturation in situ. Ongoing experiments will determine whether these cells can mature into functional FBMs.

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F-1118

A SYSTEMATIC APPROACH TO GENETIC SCREENING OF CELL PRODUCTS DEVELOPED FOR NEURAL TRANSPLANTATION

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As cell therapies advance towards clinical application, there is an imperative to ensure the safety of the transplanted cells. One of the legitimate concerns about the therapeutic grafting of the differentiated progeny of pluripotent stem cells is the possibility of graft giving rise to a malignant tumour, or the development of other forms of disease arising from the transplanted cells. In vivo grafting studies help to provide some confidence in the safety of the cell product but a number factors – xenograft vs allograft differences, relatively short lifespan of experimental animals, and disease biology – mean that this form of safety testing can never completely exclude the possibility of a deleterious consequence of the cells once transplanted in humans. Comprehensive genetic screening of a cell product offers a complementary approach to assist in reducing the risks of transplantation as much as is currently feasible. However, any comprehensive sequencing of a cell product, whether that is through whole exome, whole genome or targeted approaches, will inevitably identify numerous variants of unknown significance which may present challenging decisions regarding the use of the cells in humans. We propose a systematic method for performing the analysis of genetic screening data from neural cell products and for evaluating the risk that these variants pose in transplantation. Whilst the majority of genes of interest are oncogenic there are additional genetic mutations that would be undesirable in a cell product, many of which will be tissue-specific. As these cell therapies move towards the clinic it will be essential to define a priori which variant, or type of variant, in which gene would halt the use of the product as distinct from those variants for which the risk is negligible or below an acceptable limit. We have developed an approach using established genetic variation databases, variant pathogenicity algorithms, and guidelines drawn from the field of clinical genetics, together with mathematical risk modelling, to provide a database of genetic variants that we would consider incompatible with neural transplantation in humans.

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F-1120

LATERAL GANGLIONIC EMINENCE ISLET1 PROGENITORS CONTRIBUTE TO MATURE INTERNEURON SUBTYPES IN MOUSE NEOCORTEX

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Cortical interneurons arise from stem cells located within the ventricular & subventricular zones of the medial and caudal ganglionic eminences. However, it is unclear if these are the sole sites of origin since not all cortical interneurons have been accounted for. In the current work we present conclusive evidence that some cortical interneurons are derived from LIM homeodomain, Islet1 (Isl1) transcription factor-expressing progenitors located with the LGE. We show that Isl1 is highly expressed during neurogenesis, but is subsequently down-regulated as neuroblasts leave the LGE. Thus, we genetically fate-labeled Isl1+ progenitors and their progeny during neural development to visualize the complete lineage of Isl1-derived cells. Our studies show that Isl1 progenitors contribute to both cortical and striatal interneuron lineages. A combination of ex vivo and in vivo cell transplantation, live cell imaging, and birth-dating experiments indicated a diversification into neuronal subsets, with a bias towards parvalbumin+ fast spiking cells. Using genetic ablation of Nkx2.1+ stem cells in the medial ganglionic eminence within Isl1+ genetically fate-labeled animals demonstrates the contribution of these populations towards cortical interneuron development. Ongoing studies are investigating connectivity and function. Taken together, our results indicate that all ganglionic eminence structures contribute to neocortical interneuron diversity and that the LGE generates a small but distinct subpopulation of cortical cells.

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F-1122

SYNAPTIC INTEGRATION OF INTRASTRIATAL VERSUS INTRANIGRAL GRAFTS OF HUMAN EMBRYONIC STEM CELL DERIVED NEURONS IN THE ADULT RAT BRAIN

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Human embryonic stem cell (hESC)-derived neurons have been shown to survive long-term, release dopamine and to extensively innervate correct host structures after transplantation into adult rat brain. Using the monosynaptic tracing technique, we have recently shown that hESC-derived neurons integrate into host circuitry by establishing both host-to-graft and graft-to-host synaptic connectivity. Here we use the same tracing methodology to investigate connectivity of midbrain (MB) and forebrain (FB) patterned hESCs-derived neurons transplanted in striatum or substantia nigra. 6-OHDA lesioned rats received intrastriatal or intranigral transplants of MB-patterned and FB-patterned neural progenitors. To assess for synaptic connectivity, animals were injected with rabies vector either 17 weeks after transplantation and perfused one week later for histological analysis. We show that 18 weeks post-transplantation, host neurons from local or distant afferent structures are able to establish synaptic connections with both intrastriatal and intranigral grafts. The pattern of connectivity varied depending on the location of transplantation as intranigral grafts received more inputs from hypothalamus and midbrain nuclei while intrastriatal grafts revealed preference to thalamus and prefrontal cortex. The neuronal subtype of the graft did not have a major impact on synaptic integration, as both MB-patterned and FB-patterned transplanted neural progenitors received inputs from similar host structures. Overall, we show that both intrastriatal and intranigral grafts of hESC-derived neurons can integrate into host circuitry and that the pattern of host connectivity is dependent on the location of the transplant.

F-1124

INDUCED PLURIPOTENT STEM CELLS DERIVED FROM IDIOPATHIC PARKINSON'S DISEASE PATIENTS IMPROVED MOTOR FUNCTION OF PARKINSON'S DISEASE MODEL MONKEYS

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Induced pluripotent stem cells (iPSCs) are promising source for cell replacement therapies. In this study, we evaluated growth, differentiation, and function of iPSC-derived dopaminergic (DA) neuron progenitor in primate PD models. We generated 4 iPSC lines from 3 idiopathic PD patients and 4 iPSC lines from 4 healthy individuals using an episomal vector method. Then we produced DA neuron progenitors from these cell lines and transplanted them into brains of PD model monkeys. DA neuron progenitor from each cell line is transplanted to one monkey. MPTP-treated cynomolgus monkeys are used as primate PD model animals. We carried out 12 months observation of monkey PD scores and observed significant improvement of PD scores. Some monkeys are subjected to long-term analysis for tumor formation up to 24 months. We performed MRI analysis of monkeys at every 3 months, and observed no tumor formation. Histological analysis showed that 6.5 × 104 ± 4.9 × 104 (average ± standard deviation) tyrosine hydroxylase (TH) positive cells survived per grafts. We also performed positron emission tomography (PET) study, and the binding potential of [11C]PE2I increased after cell transplantation in some grafts, suggesting DA neuronal maturation in those grafts. In conclusion, preclinical study of cell transplantation therapy for PD revealed that DA neuron progenitors from both PD-iPSCs and healthy individuals improved PD monkey behaviors and these cells caused no tumor formation or adverse effect in the observation period.

F-1126

THE EFFECT OF TRANSPLANTED BULBAR OLFACTORY ENSHEATHING CELLS ON ENDOGENOUS STEM/PROGENITOR CELLS AFTER SPINAL CORD INJURY IN MICE

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Cell therapies have raised great hope for regenerative medicine. It has been shown that endogenous spinal cord stem cells, together with astrocytes and pericytes significantly contribute to the recovery of spinal cord injury (SCI). More recently, clinical data showed bulbar olfactory ensheathing cells (bOECs) obtained from a patient's olfactory bulb enhanced functional recovery and could be a very attractive therapeutic approach. However, the cellular mechanisms behind the effect of transplanted bOECs on spinal cord stem/progenitor cells after SCI is still poorly understood, which leads to the failure of repetition of the clinical treatments. Thus, we further study the effects of transplanted bOECs on spinal cord stem/progenitor cells after SCI. Using FoxJ1-CreERT2::YFP transgenic mice, our primary neurosphere assay showed that upon SCI, ependymal cells have higher self-renewal potential after bOECs transplantation, and their capability of differentiation to neurons is also highly up-regulated. Similarly, our in vivo fate-mapping data showed that upon SCI with bOEC transplantation, there is higher proliferation and migration to the lesion site of ependymal cells. More interestingly, we unexpectedly discovered enhanced adult neurogenesis after bOECs transplantation upon SCI. In addition, we used FoxJ1-Rasless mice to specifically block the cell cycle of ependymal cells, we found that after transplantation, the spinal cord self-repair capacity by astrocytes is highly recruited compared to vehicle, and ependymal cells are required together with bOECs to promote adult neurogenesis after SCI.Altogether, we here describe the transplanted OECs can better activate endogenous stem cell potential after SCI and promote adult neurogenesis in the spinal cord. Moreover, bOECs transplantation promotes the self-repair capacity of endogenous glial cells, which suggests transplantation of bOECs to injured spinal cord would be a beneficial therapeutic possibility for the recovery after SCI.

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F-1128

THE KYNURENINE PATHWAY OF TRYPTOPHAN METABOLISM MODULATES NEURAL STEM CELL PROLIFERATION

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The search for molecules which critically regulate neural stem cell (NSC) proliferation is ongoing, and underpins future production methods of specific lineages of committed cells for cell therapy, while also contributing to understanding why innate repair mechanisms in neurodegenerative diseases may fail. Our research over many years has investigated a role of the kynurenine pathway (KP) in healthy metabolism and in neurodegenerative disease. The KP plays a critical role in regulating bioavailability of the essential amino acid tryptophan (Trp). Sequentially metabolised, ultimately the essential co-factor nicotinamide adenine dinucleotide (NAD+) is produced. In diseases like Multiple Sclerosis the KP is dysregulated, producing high levels of metabolites like Quinolinic acid, which we've shown is highly toxic to brain cells. In this work, we aimed to investigate if modulating the KP altered NSC proliferation. In particular, to confirm if interferons (IFNs) act as molecular triggers to activate the KP and drive changes in the proliferation of NSCs. Developing mouse NSCs commercially obtained from E14 neurospheres were cultured in the presence of EGF. Agonists, antagonists or siRNAs to KP enzymes were used to dissect the pathways. IFN-γ is a known activator of indoleamine-2,3-dioxygenase (IDO-1) expression, the initial rate-limiting enzyme metabolising Trp, and indeed significantly induced IDO-1 in NSCs. NSCs express all other KP enzymatic machinery, and IFN-γ lead to impaired proliferation and an alteration of the metabolic state of NSCs including their NAD+/NADH ratio (representing cell energy levels). This effect is through Trp depletion (required for protein biosynthesis), rather than through the effects of KP metabolites. In contrast IFN-β had a negligible effect on IDO-1 levels, but induced IDO-2, and significantly decreased proliferation and downstream

enzyme kynurenine-3-monooxygenase. In conclusion, we provide the first evidence that KP enzymes play a specific role in the biology of NSCs and tryptophan metabolism, including the dominant regulation of the KP by the interferons such as IFN-γ and IFN-β. This suggests that selective KP inhibition could minimize cell death during inflammatory episodes and optimize NSC proliferation and differentiation with direct therapeutic applications.

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NEURAL DISEASE AND DEGENERATION

F-1132

DOUBLECORTIN DURING EARLY NEUROGENESIS

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Human doublecortin (DCX) mutations cause Lissencephaly; a genetic developmental brain disorder characterized by severe cortical malformations including smooth cerebral surface and a thickened cortex with neurological impairments such as seizures, and intellectual disability. Mutant cells have large effects on neuronal cell migration leading to aberrant neuron positioning giving rise to an unorganized cortex with severe brain malformations. In the absence of comparable animal models and limited patient brain material available, we took advantage of induced pluripotent stem cell (iPSC) technology and have developed a distinct in vitro neural cell model to study the molecular mechanisms caused by the DCX mutation. To derive disease relevant cell types we have reprogrammed fibroblast cells from 2 Lissencephaly patients with mutated DCX gene and 2 healthy individuals. The disease was recapitulated by directing the iPSCs into long-term neuroepithelial stem cells (NESCs) and further differentiation to neuronal cells followed by expression profiling and dissection of DCX associated functions. Here we focused on the transcriptome profile of NESCs and differentiating neurons at different time points to investigate perturbed gene expression, comparing DCX mutant with controls. We found that the gene expression profile at NESC stage from both the mutated cells and control cells are highly similar when DCX is not yet expressed. Interestingly, the difference between groups was observed after 7 days of differentiation, which coincided with up-regulation of DCX, suggesting that transcriptional profiles correlated to the cell line's DCX-linked Lissencephaly background. Moreover, bioinformatics analysis indicated several genes linked to cell adhesion, cell proliferation, cell migration, and neuronal development were de-regulated in DCX mutant cultures compared to controls. Our data so far indicates that in addition to a key role in cell migration, DCX is also significantly impacting on other cellular processes as well as neuronal networks.

F-1134

USE OF IPSC-DERIVED HUMAN NEURONS IN QUANTITATIVE HIGH-THROUGHPUT SCREENING

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Human neurons differentiated from induced pluripotent stem cells (iPSCs) represent an opportunity to model diseases of the nervous system in a more relevant way and afford a better in vitro platform for drug discovery. However, until recently, their use in high-throughput screening (HTS) has not been feasible. Toward this end, we have developed techniques to produce highly enriched (>90% purity) human iPSC-derived neurons in batch sizes of greater than one billion cells. We have also addressed some of the practical challenges of using human neurons for screening, such as plating by automated dispenser and use of low reagent volumes. Importantly, a specialized rapid maturation supplement has been developed to accelerate their maturation to allow screening experiments to be completed in three to four days. We have employed this approach to screen compounds in two motor neuron reporter systems for amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Using genome editing techniques, nanoluciferase (Promega) fusion reporters were inserted into patient iPSCs to monitor the levels of endogenous neurofilament light chain (NF-L) for ALS and full-length survival motor neuron 2 (SMN2) for SMA. Applying a quantitative HTS approach, we screened the LOPAC, NPC, and MIPE libraries (~6,000 compounds) in a dose dependent manner on both motor neuron lines with a hit rate of ~0.5%. These hits were validated in 96-well format using freshly prepared compounds. We are currently screening the NIH Genesis library to discover novel compounds that increase SMN2 protein levels. This library contains ~100,000 compounds representing ~1,000 unique scaffolds, allowing us to interrogate a large and diverse chemical space. Overall, this program

demonstrates the feasibility of using iPSC-derived human neurons to conduct HTS with a platform more relevant to diseases of the human nervous system.

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F-1136

WINDOWS OF SUSCEPTIBILITY FOR ZIKA VIRUS INDUCED MICROCEPHALY IDENTIFIED BY TEMPORAL GENE ANALYSIS OF A PLURIPOTENT STEM CELL MODEL

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Increased understanding of developmental disorders of the brain has shown that genetic mutations, environmental toxins and biological insults typically act during developmental windows of susceptibility. Through analysis of developmental time-course gene expression data derived from human pluripotent stem cells, with disease association, pathway, and protein interaction databases, we identify windows of developmental time that appear most vulnerable to a specific insult, and therefore, the time periods for productive interventions. The results are displayed as interactive Susceptibility Windows Ontological Transcriptome (SWOT) Clocks illustrating disease susceptibility over developmental time. Using this method, we determine the likely windows of susceptibility for multiple neurological disorders, including Zika-induced microcephaly. We find that genes impacted by Zika infection are most active in the earliest stages of neural development, prior to cerebral cortex layer formation.

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F-1138

MODULATION OF ASTROGLIOSIS IN PLURIPOTENT AND MULTIPOTENT STEM CELLS WITH GSK-INHIBITORS AS A POTENTIAL TREATMENT OF SPINAL CORD INJURY

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Spinal cord injury (SCI) results in neural loss and consequently motor and sensory deficit below the injury. Recently, we have reported the regenerative effects and significant improvement of locomotor function in complete transection rat model of SCI following transplantation of oligodendrocyte progenitors cells (OPC) and motoneuron progenitors (MP) derived from hESC. Transplantation of these progenitors promote astrogliosis, thorough activation of jagged1-dependent Notch and Jak/STAT signalling supporting axonal survival. Induction of astrogliosis and neurogenesis can be achieved by inhibition of glycogen synthase kinase-3 (GSK3) well known molecule involved in several signalling pathways. In the present study we assess the in vitro effects of Ro3303544 (more potent and less toxic GSK-3 inhibitor, Ro) using ependymal stem cells as well as hESC and hiPSC-derived neural progenitors. Our result show significant increase of astrogliosis (GFAP) and neurogenesis (Tuj1 and MAP2) in the cells treated with Ro compared to untreated cells during 3 days of treatment. This study will contribute to the discovery of new combined therapies including transplantation of pluripotent stem cells derivates with augmentation of protective functions of reactive astrocytes which may lead to novel approaches to reducing secondary tissue degeneration and improving functional outcome after SCI.

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F-1140

CONDITIONAL KNOCK OUT BCAS2 IN ADULT FOREBRAIN CAUSING NEURAL DEFECT CAN BE IMPROVED BY LITHIUM TREATMENT

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Breast carcinoma amplified sequence 2 (BCAS2) is a core component of the hPrP19 complex that controls RNA splicing. BCAS2 is an upstream regulator for controlling β-catenin gene expression. Previously, we generated conditional knockout (cKO) mice to eliminate the BCAS2 expression in forebrain that show a microcephaly-like phenotype with impaired learning and memory coupled with dendritic malformation. The mechanism is that BCAS2 can regulate dendrite growth at least one role through β-catenin. Here, we further investigated lithium therapeutic effect on BCAS2 cKO. Lithium, an inhibitor of glycogen synthase kinase-3β (GSK-3β), leads to the activation of the Wnt/β-catenin signaling pathway and an increase in β-catenin. Firstly, we measured filopodia formation in lithium treatment of N2A cell; the results revealed that lithium treatment could increase β-catenin expression along with increased numbers of filopodia in BCAS2 knockdown-differentiated N2A cells. We then further examined whether lithium could rescue BCAS2 cKO-causing neural degeneration. The reactive gliosis in cKO mice was measured; and showed high Iba1 exression in dentate gyrus molecular layer (DG Mo) and lithium treatment could eliminate Iba1 expression in DG Mo. Additionally, lithium treatment could improve their spatial learning and memory capability of BCAS2 cKO mice coupled with increased β-catenin expression in cortex and hippocampus both in WT and cKO mice. These results further support that BCAS2 regulates cognitive learning and memory through β-catenin. On the other hand, Wnt/β-catenin signaling reportedly regulates adult hippocampal neurogenesis. Our results showed cKO exhibited declined number of Sox2-positvie NSCs those were restored by lithium treatment. Taken together, BCAS2 can regulate neuron stem cell activation/proliferation; lithium treatment can improve cognitive learning and memory and rescue Sox2 stem cell activation/proliferation in BCAS2 cKO mice.

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F-1142

APPLICATION OF HUMAN PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS (IPSCS) TO STUDY THE ROLE OF NEURITE INHIBITION AND MECHANISMS OF RECOVERY IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a complex neurodegenerative disorder that leads to a clinical decline in neurological function due to a loss of neuronal connectivity in the brain. The accumulation of extracellular β-amyloid (Aβ) peptide in the form of senile plaques is a hallmark of the disease and is thought to result in morphological abnormalities in developing and regenerating neurites. An increase in the ratio of the 42 amino acid long Aβ species (Aβ42) compared with the 40 amino acid long Aβ species (Aβ40) is associated with the deposition of Aβ in AD as insoluble fibrils within senile plaques. In this study we harness induced pluripotent stem cell (iPSC) technology to investigate the role of Aβ in suppressed neurite outgrowth in AD, through the culture of iPSC derived neuroprogenitor cells originating from a healthy individual (ReproNeuro), an AD patient (ReproNeuro AD-patient 1) with a mutation in presenilin 2 or cells transfected with an AD associated mutation (ReproNeuro AD-mutation) in presenilin 1. These AD phenotype iPSC-derived neuroprogenitor cells are associated with a higher Aβ42:Aβ40 due to their respective mutations in presenilin, a component of the γ-secretase complex that is responsible for the cleavage of amyloid precursor protein (APP) and the formation of Aβ species. We have discovered an impaired ability of AD phenotype iPSC-derived cells to generate neurites in a novel 3D neurite outgrowth assay and have screened the ability of molecules to recover such inhibition. Inhibitors of the Rho A signaling pathway are commonly used in neurite outgrowth cultures to promote the elongation of neurites and may potentially be used to restore neurite outgrowth in the presence of an inhibitory stimulus. We have assessed the ability of ibuprofen, a non-steroidal anti-inflammatory drug (NSAID) and inhibitor of Rho A, alongside the ability of the selective ROCK inhibitor Y-27632, to overcome neurite inhibition in this novel 3D culture system. We have found that inhibition of both Rho A and ROCK can restore neurite outgrowth in AD phenotype cells to control levels. This novel in vitro assay based on iPSC and 3D cell culture technology offers new exciting opportunities for the research and discovery of pharmaceuticals to overcome the inhibition of neurite formation in neurological disorders such as AD.

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F-1144

NEURODEVELOPMENTAL DIFFERENCES UNDERLIE DISCORDANT PSYCHOSIS IN A PAIR OF MONOZYGOTIC TWINS

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Despite extensive studies, the cellular basis of schizophrenia (SZ) and bipolar disorder (BP) is still not elucidated. The concordance rates of SZ and BP in monozygotic twins are much higher than in dizygotic twins, but not 100%, suggesting a contribution of both genetic and epigenetic factors. iPSC technology is expected to provide clues for understanding the cellular basis of these disorders. However, identification of subtle disease-associated cellular phenotypes is complicated by large genetic variability between individuals. Analyzing discordant monozygotic twins is the ideal way to detect subtle differences caused by the disease. Here, we generated iPSCs from a pair of monozygotic twins discordant for psychosis (schizoaffective disorder). Neuronal differentiation potency of iPSCs did not differ between the twins, whereas the length of neurites extended from neurospheres were different between the twins. Moreover, electrophysiological analyses suggested an altered balance of excitatory and inhibitory synaptic transmission in the affected twin-derived cells compared to the co-twin. Consistent with the cellular phenotype, RNA-seq analysis of neural stem/progenitor cells found significant differences in the expression of genes involved in axonal guidance signaling. Additionally, the expression pattern of genes related to Wnt-signaling pathway and GABAergic neuronal differentiation were significantly different between the twins. To further study the phenotypic consequence of the altered Wnt/β-catenin signaling in the affected twin, we modeled neurodevelopment using cerebral organoids. Compared with the co-twin, the affected twin-derived organoids showed smaller cortex-like structures and disrupted alignment of proliferative NSCs along the apical surface. However, activation of Wnt/β-catenin signaling with GSK3β inhibitors improved the phenotype of the patient's organoids. Altogether, these results suggest a possibility that differences during early neural development associated with diminished Wnt/β-catenin signaling activity underlie the discordant psychosis between the monozygotic twins.

F-1146

SCREEN FOR SMALL MOLECULES THAT PROMOTE MYELINATION OF HUMAN STEM CELL DERIVED OLIGODENDROCYTES

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Multiple Sclerosis, a neurodegenerative disease whose pathogenesis involves immune-mediated demyelination, is a leading cause of optic neuritis, in which demyelination of the optic nerve causes acute, and potentially chronic, visual loss. Currently, there are no clinically approved therapeutic approaches that directly promote neuronal remyelination and/or neuronal survival. As an approach to developing such therapies, we are working to establish an effective in vitro myelination assay that works with human cell culture systems, with the goal of performing a high-content screen of small molecule libraries to identify compounds that promote myelination, with the ultimate goal of using such molecules as leads to aid in the development of re-myelination strategies to complement current approaches for MS treatment. Using CRISPR/Cas9, we have successfully generated a dual knock-In reporter stem cell line that expresses tdTomato driven by the PDGFRa promoter, which should also express GFP when myelin basic protein (MBP) is expressed. After differentiation the PDGFRa+/tdTomato+ reporter oligodendrocyte precursor cells (OPCs) can be purified using either FACS sorting or immunopurifcation. We have successfully used this strategy to develop reporters for stem cell-derived retinal ganglion cells (RGCs) that expresses tdTomato driven by the RGC specific gene POU4F2/BRN3B. With our OPC differentiation protocol O4+ OPCs are detected as early as day 60 and MBP+ oligodendrocytes (OLs) within 100 days of differentiation, and tdTomato+/POU4F2+ RGCs are detected by day 25 in the RGC reporter lines using our RGC protocol. After differentiation we can purify PDGFRa+/tdTomato+ OPCs and tdTomato+/POU4F2+ RGCs with 90% and 95% purity, respectively. We plan to co-culture purified RGCs and purified OPCs to establish an in-vitro myelination system. Either the co-culture or the immunopurified OPCs will then be plated into a 384-well or a 1536-well culture format for high-throughput screening.

F-1148

SYNTHETIC LETHALITY SCREENING IN ISOGENIC HUNTINGTON DISEASE NEURAL PROGENITOR CELLS TO IDENTIFY MODULATORS OF MUTANT HTT LOSS AND GAIN OF FUNCTION EFFECTS

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Huntington disease (HD) is a fatal neurodegenerative disorder caused by an expanded polyglutamine tract in the huntingtin (HTT) protein. Mutant HTT causes cellular dysfunction through mechanisms involving toxic gain- and loss-of-function effects. Pathways and processes disrupted by mutant HTT include transcription, mitochondrial metabolism and proteasomal degradation. However, currently there is no effective therapy for HD, and a significant challenge is the identification of cellular targets for therapeutic intervention. To enable the rapid screening of genes or pathways that play important roles in the survival of HD neural cells, we have generated an isogenic allelic panel of human HD embryonic stem cells (IsoHD lines) by genome editing with TALEN technology. Neural progenitor cells were derived from IsoHD lines with 30 CAG repeats (control) and 81 CAG repeats (mutant) in the HTT gene. We next performed a large-scale dropout screen in these cells using two pooled shRNA libraries each comprising 27000 shRNA targeting 5000 genes. The genes targeted by the two libraries are involved in signaling pathways and disease related pathways, respectively. The shRNA library interference specifically reduce the levels of individual cellular proteins both in control and HD neural progenitor cells. By checking the shRNA sequence from the drop-out cells using next generation sequencing, we obtained a list of genes that protect the cells from mutant htt insults. We also generated stable HTT knockdown cell lines in human fetal neural progenitor line ReNCell with 70% HTT protein knocked down shown by western blot. Large-scale shRNA dropout screening was similarly applied in these cells to identify genes that are critical for the survival of neural progenitor cells when HTT is absent. Combined, our two screening strategies enable the identification of potential key modulators of mutant HTT gain- and loss-of-function effects.

F-1150

ESTABLISHMENT OF INDUCED PLURIPOTENT STEM CELLS FOR MODELING OF ALZHEIMER'S DISEASE

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Alzheimer's disease(AD) is a progressive neurodegenerative diseases and accounts for 60 to 80 percent of dementia cases. However, the pathogenesis of AD is far from clear because of the difficulties in obtaining live neurons from patients and the inability to model the disease. The progression of induced pluripotent stem cell (iPSC) techniques may make it possible to establish a cellular model of AD. Here we reprogrammed human peripheral blood mononuclear cells (PBMCs) from AD patients and non-demented control individuals into iPSC lines using footprint-free technology. PBMCs were isolated from 8 healthy control subjects and 17 AD patients and iPSCs were generated using the CytoTune®-iPS Sendai Reprogramming Kit following the manufacture's protocol. The expression of pluripotency markers (OCT4, SSEA4, TRA-1-60, NANOG) was detected and visualized by immunofluorescent staining using specific antibodies. Karyotype analysis was performed to ensure the stability of genetic materials in iPSCs. iPSCs were differentiated into neural stem cells(NSCs) using STEMdiff™ Neural Induction Medium (Stem Cell Technologies). The neural specific marker Nestin was stained and detected by flow cytometry using specific antibody. Targeted exome sequencing of APP, PSEN1 and PSEN2 were conducted to detect mutations or Single-Nucleotide Polymorphisms (SNPs) in these genes. Chi-square test in was used for statistics analysis. We obtained 14 and 30 colonies of iPSCs respectively by reprogramming PBMCs from control subjects and AD patients. More than 90% iPSCs expressed the stem cell marker Oct4, Nanog, Sox2 and SSEA4. Karyotype analysis confirmed that iPSCs carry a normal karyotype during the process of generations. More than 95% iPSC-derived NSCs expressed Nestin during the subculture for 5 generations. A C261T single nucleotide variant (SNV) in PSEN2 was detected in 11 AD patient-derived and 8 healthy subject-derived iPSCs. However, chi-square test showed that there was no difference in the C261T SNV frequencies of PSEN2 between normal control and AD patients. No mutation or SNP was detected in normal control nor AD patients in gene PSEN1 or APP. In conclusion, these results suggest that we successfully generated iPS cells from PBMCs of AD patients and these iPS cells may be useful for modeling AD.

F-1152

TRANSCRIPTIONAL PROFILING OF AN IPSC-BASED NEURONAL MODEL OF X-LINKED DYSTONIA-PARKINSONISM

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X-linked Dystonia Parkinsonism (XDP) is a neurodegenerative disorder endemic to the Philippines that was linked to a founder haplotype over two decades ago. This haplotype contains seven non-coding disease-specific changes (DSCs) within or distal to the TAF1 gene, which include 5 missense mutations, a 48-bp deletion, and a 2.4-kb sine-VNTR-Alu (SVA) retrotransposon insertion. However, it is not yet understood how any of these mutations contribute to disease pathogenesis, in part due to the dearth of relevant disease models. In this study, we performed transcriptome-wide analyses on neuronal cells generated from patient-derived induced pluripotent stem cells (iPSCs) to identify pathogenic mechanisms associated with XDP. RNA-seq uncovered alterations to genes, pathways, networks, and co-expression modules of relevance to both dystonia and Parkinsonism, as well as a co-expression network strongly enriched for interactions previously implicated in transcriptional regulation and neurodevelopmental disorders. Furthermore, differential gene expression and de novo transcriptome assembly revealed novel alternative splicing and partial exonization of intronic sequence as a consequence of retrotransposition into the XDP founder haplotype. Remarkably, both the aberrant splicing and reduced canonical TAF1 expression signatures in XDP probands were rescued following CRISPR/Cas9 excision of the SVA. These data suggest a unique genomic cause of this unsolved Mendelian disorder, and may be used to guide future studies that seek to elucidate XDP disease pathogenesis.

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CANCERS

F-1158

CLONAL ANALYSIS DEFINES THE HIERARCHICAL MODE OF MEDULLOBLASTOMA GROWTH AND A REQUIREMENT FOR COMBINATION THERAPY IN A PRE-CLINICAL MOUSE MODEL

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Functional heterogeneity within tumors presents a significant therapeutic challenge. We have previously shown that the pediatric brain tumor medulloblastoma (MB) contains a rare fraction of quiescent cells defined by expression of the transcription factor Sox2. At the population level, Sox2+ cells give rise to the increasingly differentiated and proliferative cell types comprising the tumor bulk and are selectively enriched following treatment with anti-mitotics or Sonic Hedgehog (SHH) pathway antagonists. These findings suggest Sox2+ cells are a likely source of post-treatment disease relapse, which is almost always fatal in patients. We hypothesized that these cells drive tumor progression through hierarchical growth and that their elimination will be critical in order to achieve full disease cure. To test this, we first performed a clonal-level lineage trace from the Sox2+ fraction with a multi-coloured fluorescent reporter. Quantitative modelling of clonal fate data conformed to a hierarchical mode of growth, with Sox2 expression defining a population of long-lived, self-renewing cells. Our modelling also predicted that the rapidly dividing progeny of Sox2+ stem cells represent a self-renewing progenitor fraction that gives rise to a terminally differentiated cell compartment. To functionally probe the reliance on Sox2+ cells for overall tumor progression, we utilised a conditional ablation model to selectively kill Sox2+ cells in tumor bearing mice and found no difference in a survival assay. This finding suggests that the progenitor fraction is capable of driving disease progression in the absence of their Sox2+ parent cells. Demonstrating the utility of a combination approach to circumvent this, we ablated the Sox2+ fraction alongside treatment with a SHH pathway antagonist (targeting the rapidly dividing progenitor cells) and observed a concordant delay in tumor progression. In order to translate this proof-of-principle experiment into a pre-clinical setting, we have probed the mechanisms driving self-renewal of the Sox2+ cell fraction through both CHiP-Seq and ATAC-seq on isolated Sox2+ cells. Analysis of these

data sets have revealed potential targets that could be therapeutically exploited in combination with SHH antagonism.

F-1160

SOX2, A STEMNESS MARKER, IS RELATED WITH DRUG RESISTANCE IN NON-SMALL CELL LUNG CANCER CELLS

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There are two types of lung cancer, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC accounts for about 85% of all lung cancers. Chemotherapy is one of the most common treatments for cancer, however, chemoresistance is a major hurdle in successful cancer therapy. Unfortunately, the mechanisms of chemoresistance are not yet fully elucidated. In order to investigate this problem, recent studies commonly utilize three-dimensional (3D) cell culture models. 3D cell culture facilitates the formation of spheroids or aggregates whose are more analogous to their form in vivo. It is also well known that chemoresistance is related to cancer cell stemness. Here, we compared NSCLC cultured in two-dimensional (2D) system with those cultured in 3D system and examined drug resistance in 3D culture model. For this, NSCLC cell A549 was cultured in two groups: (i) as monolayers attached to plastic culture dish (control), (ii) as suspended on poly (2-hydroxyethyl methacylate) (polyHEMA) coated dish. There were grape-like aggregates on the polyHEMA-coated dish. We compared the gene expression of cell cycle, apoptosis, epithelial-mesenchymal transition (EMT) and stemness markers between the two groups and focused on the stemness markers (Oct4, Sox2, Klf4, c-Myc and Nanog). Stemness markers were upregulated in the polyHEMA group compared to the control group. Indeed, A549 cells under the polyHEMA culture were resistant to vinblastine compared to A549 cells under the control culture. Recent studies show that drug resistance is caused by expression of pluripotency markers, Oct4, Nanog and Sox2. Vinblastine treatement resulted in changes of mRNA expression associated with stemness. In fact, expression level of Oct4, Klf4, c-Myc and Nanog decreased while expression level of Sox2 increased in the polyHEMA group when compared with the control group. The expression of phosphorylated Akt also increased in the vinblastine treated polyHEMA group. In summary, these findings indicate that cells detached from culture substratum make aggregates, the mimic form of solid tumor, and induce stemness and development of drug resistance. Drug resistance is mediated by upregulation of Sox2 dependent on the PI3K/Akt pathway. Sox2 may become a marker of drug resistance in NSCLC prognosis.

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F-1162

HYPOXIA-NOTCH1-SOX2 SIGNALING IS IMPORTANT FOR MAINTAINING CANCER STEM CELLS IN OVARIAN CANCER

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Ovarian cancer stem cells (CSCs) are characterized by the ability to form spheres in culture, overexpression of stemness marker genes, including SOX2, ABCB1, ABCG2, and ALDH, and resistance to chemotherapeutic drugs. Hypoxia and NOTCH signaling have been reported to play important roles in maintaining stemness of CSCs. However, the relationship between hypoxia and NOTCH signaling has not been evaluated in ovarian CSCs. Ovarian CSCs were enriched through sphere culture of ovarian cancer cells and SOX2 was identified as a critical factor for maintaining CSC-like characteristics. Hypoxic treatment of ovarian cancer cells resulted in enhanced sphere formation and expression of NOTCH1, SOX2, and ALDH1. Overexpression of intracellular domain of NOTCH1 (NICD1) increased SOX2 and ALDH1 expression in ovarian cancer cells. SOX2 promoter activity was up-regulated by hypoxic treatment or NICD1 overexpression. In addition, deletion of RBPJκ binding sites resulted in elimination of hypoxia-induced up-regulation of SOX2 promoter activity. Knockdown of SOX2 resulted in inhibition of hypoxia- or NICD1-induced enhancement of CSC characteristics including sphere formation and resistance to chemotherapeutic reagent. These results suggest that hypoxia-NOTCH1-SOX2 signaling axis plays an important role in ovarian CSCs, which may provide a novel opportunity for developing therapeutics for eradication of CSCs in ovarian cancer patients.

F-1164

INCREASED SENSITIVITY OF ANEUPLOID EMBRYONIC STEM CELLS TO GENOTOXIC STRESS

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Aneuploidy, an incorrect number of chromosomes, leads to severe developmental defects and is also a hallmark of cancer. The cellular consequences of aneuploidy are largely dependent on the specific cell types. Trisomic yeast and mouse embryonic fibroblasts (MEFs) exhibit cell proliferation defects, while some aneuploid embryonic stem (ES) cells and induced pluripotent stem (iPS) cells proliferate rapidly and reach higher saturation densities. Because the previously reported aneuploid yeast and MEFs showed increased sensitivity to several compounds, including the energy stress-inducing agent AICAR (aminoimidazole carboxamide ribonucleotide), the protein folding inhibitor 17-AAG (17-(Allylamino)-17-demethoxygeldanamycin), and the autophagy inhibitor chloroquine, we want to test whether there is proteotoxic, energy and genotoxic stress in aneuploid ES cells by using our established trisomic ES cell lines, including trisomy 6, 8, 11, or 15. Cell viabilities were detected after the exposure of wild-type and trisomic ES cells to the compounds that cause proteotoxic stress (cycloheximide, MG132, 17-AAG, chloroquine), energy stress (AICAR), or genotoxic stress (adriamycin, methyl methanesulfonate, hydroxyurea, mitomycin C, etoposide). Unlike aneuploid yeast and MEFs, nearly all trisomic ES cell lines could not be selectively inhibited by compounds that cause proteotoxic or energy stress. These findings are in line with the previous works about human ES cells with trisomy 21 or trisomy 12. Meanwhile, most of the trisomic ES cells showed increased sensitivity to compounds that cause genotoxic stress, which was further confirmed by colony forming experiment, and the levels of apoptosis and DNA damage response proteins were also detected. In conclusion, our results demonstrate that aneuploidy may affect the maintenance of genomic stability, and then facilitate malignant cell growth.

F-1166

HAPLOID HUMAN EMBRYONIC STEM CELLS IN PERSONALIZING CHEMOTHERAPEUTIC TREATMENT

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Innate resistance to chemotherapies is a major concern in first-line chemotherapy treatments. It is well established that this resistance is rooted in genetic predispositions that prevent the chemotherapy used from being effective in diminishing the cancerous cells. Still, no comprehensive strategy is being offered today for personalizing the chemotherapy treatment to the specific resistance profile of the tumor. Here, we used haploid human embryonic stem cells (ESCs) in order to establish a comprehensive transposon-based loss-of-function library, targeting genes that are expressed in human ESCs. This loss-of-function library was utilized for identifying genes that confer resistance to chemotherapies such as bleomycin. Resistance genes showed enrichment for the cellular pathways unique to the chemotherapy's mechanism of action. In bleomycin screening, we identified the DNA damage related gene RIF1 as highly mutated, and confirmed its involvement in bleomycin resistance by down-regulating it in normal diploid cells. These results suggest that alterations in DNA-repair mechanism are involved in the resistance against bleomycin. Such genome-wide analyses can be extended in order to further delineate the involvement of resistance genes in chemotherapy resistance for advancing personalized medicine in cancer.

F-1168

DEVELOPING STRATEGIES TO TARGET AND KILL CANCER CELLS EXPRESSING THE WNT RECEPTOR FZD7

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Cancer stem cells (CSC) are rare cells within a tumor that fuel the growth of a cancer, and eradication of cancer requires that these rare CSCs are specifically targeted and killed. However, the lack of CSC-specific markers has made it difficult to target and kill these cells. We have identified a cell surface marker, encoded by the FZD7 gene, which is highly expressed in several human cancers, including breast, ovary, and brain. Our previous

research established an essential function for FZD7, a Wnt receptor, in human pluripotent stem cells. To further elucidate the function of FZD7 in stem and cancer cells, we have developed a highly specific antibody, which potently inhibits Wnt signaling activity and disrupts the pluripotent state. Because FZD7 expression is largely restricted to early developmental processes and is almost completely absent from normal adult cells, its expression in cancer makes it an ideal marker to identify and target CSCs. Using our unique FZD7 antibody, we are developing several strategies to attack cancer cells expressing FZD7. First, we have found that the antibody interferes with several properties of cancer cell lines, including proliferation, migration and clonogenicity. We are currently using this antibody to test whether FZD7 marks CSCs. Second, using standard antibody engineering methods, we generated a single chain antibody (scFv), which we have fused to a chimeric antigen receptor (CAR) that will be expressed in NK- and T-cells. These immune cells expressing the FZD7-specific CAR will be used to target human cancer cells in vitro and in vivo. The overall goal of these ongoing studies is to develop highly potent immunotherapies to target and kill cancers expressing FZD7.

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F-1170

PAPILLARY THYROID CARCINOMA PRIMARY CELL CULTURE DERIVED ALDH NEGATIVE SPHERE CELLS BEHAVE AS CANCER STEM LIKE CELL

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Papillary thyroid carcinoma (PTC) accounts for 80-85% of all thyroid cancer cases. Although most PTC patients can be treated successfully and have an excellent prognosis, 10-20% of patients with stage I or II PTC have recurrence, developing invasive tumors and/or distant metastases. To treat these patients effectively, it is necessary to have greater understanding of the mechanisms that govern PTC tumor initiation and progression. It is becoming evident that thyroid tumors may follow the cancer stem cell model (CSC), where a population of cancer stem-like cells is responsible for tumor initiation and progression. Currently, the biological properties of PTC CSC are still poorly understood. Focusing on tumor specimens collected during patient surgical procedures, we isolated sphere-growing cells from PTC-derived primary cells in stem cell growth condition. The sphere cells could be maintained in serum-free culture. In vivo transplantation revealed that the sphere cells were capable of generating xenograft tumors that recapitulated the original tumor phenotypes. Analyzing the sphere cells for aldehyde dehydrogenase (ALDH) expression, a marker commonly used in thyroid CSC study, revealed that around half of the sphere cells did not express ALDH. When sorting the sphere cells into ALDH- and ALDH+ subpopulations, both subpopulations were able to re-initiate subspheres in vitro and generate serial xenograft tumors in vivo with the ALDH+ cells exhibiting a slightly higher efficiency of sphere-formation and cancer-initiation. Of major importance, the unsorted sphere cells demonstrated the highest level of cancer-initiating cell frequency, and quicker tumor progression as xenografts when compared to the sorted ALDH- and ALDH+ cells. Furthermore, histological investigation of xenograft tumors generated by the sphere-sorted cells and the unsorted cells, respectively, revealed similar morphological features that recapitulated the characteristics of original human PTC from which the sphere cells were isolated. Our data established a vital role of ALDH- sphere-growing cells and suggested potential interactions between ALDH- and ALDH+ cells during tumor initiation and progression. Further studying the biology of ALDH- sphere cells may provide novel therapeutic solutions to treating aggressive PTC.

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F-1172

GPR133, AN ORPHAN ADHESION G PROTEIN-COUPLED RECEPTOR, PROMOTES HYPOXIA-DRIVEN TUMOR PROGRESSION IN GLIOBLASTOMA

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Glioblastoma (GBM) is an incurable primary brain malignancy. Tumor progression, as well as resistance to current therapies, is mainly attributed to glioblastoma stem cells (GSC). A hallmark of GBM is the intratumoral gradient of oxygen tension, including poorly vascularized regions of hypoxia. Hypoxia has been shown to promote the glioblastoma stem cell (GSC) phenotype and thereby lead to tumor progression. Attempts to therapeutically target Hypoxia Inducible Factors (HIFs), which mediate most of the hypoxia response, have to date been unsuccessful. We recently found that GPR133 (ADGRD1), an orphan adhesion G protein-coupled receptor (GPCR), is enriched in CD133+ GSCs. Immunohistochemistry in GBM biospecimens hows GPR133 expression specifically in the hypoxic areas of pseudopalisading necrosis, but not in normal brain. Subjecting patient-derived GBM cell cultures to hypoxia led to upregulation of GPR133 transcript (6 out of 8 cultures), while HIF1 knockdown decreased the level of GPR133 transcript ($P < 0.05$). ChIP-qPCR confirmed direct binding of HIF1 to a Hypoxia Response Element (HRE) 517 bp upstream of the GPR133 transcriptional start site ($P < 0.001$). These findings support the idea that GPR133 is upregulated in hypoxia via direct transactivation by HIF. Functionally, GPR133 knockdown decreased the CD133+ GSC population and tumor cell proliferation in vitro under both normoxic and hypoxic conditions ($P < 0.05$). Further, GPR133 knockdown impaired sphere formation in extreme limiting dilution assays ($P < 0.001$), suggesting an effect on "stem" properties. In vivo, the knockdown essentially abolished tumor xenograft initiation ($P < 0.002$) and prevented the death of mouse hosts (n=4 mice/group; $P < 0.01$), indicating a robust role for GPR133 in tumor initiation. TCGA data from 160 GBM patients revealed that higher GPR133 mRNA levels correlate with worse survival (P=0.0062). RNA-sequencing followed by gene ontology analysis revealed that knockdown of GPR133 has effects on cell cycle regulation, stem-related pathways, telomere maintenance and DNA damage response. We believe that GPR133 plays an important pro-tumorigenic role in hypoxia-driven GBM progression and it represents a promising and novel therapeutic target.

CHROMATIN AND EPIGENETICS

F-1174

EPIGENETIC ROADMAP FOR TRANSCRIPTIONAL SILENCING: A TALE OF TWO X'S

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Stem cell differentiation and loss of naïve pluripotency are linked to dynamic changes on both the transcriptomic as well as epigenomic level. Specifically, rapid gene silencing events are associated with dynamic alterations to the pattern of histone modifications eventually leading to heterochromatin formation. However, functional relevance and exact spatio-temporal dynamic of such processes remains elusive. Indeed, whether changes in chromatin are a cause or consequence of changes in gene expression is a fundamental question. To address this question we explore the functions of chromatin modifications in random X chromosome inactivation (XCI) occurring in female mouse stem cells. This process is dependent on coating of one X chromosome by a long non-coding RNA, Xist, and results in dramatic remodelling of the chromatin. Here we report an extensive epigenetic dataset of allele-specific ChIPseq experiments mapping seven chromatin marks at the initiation stages of XCI. Interestingly, we observe that the rate of inactivation is not equivalent throughout the X chromosome as some clusters of loci show rapid loss of active histone marks while others remain more resistant. What is more, we report striking differences between histone marks in their reprogramming kinetics. This allowed us to map the temporal hierarchy of epigenetic events occurring as early as 4 hours post Xist expression. By combining ChIPseq datasets with transcriptomic analysis, we

uncover that loss of some active epigenetic marks is tightly linked to gene repression. We further show the functional relevance of such modifications by employing CRISPR/Cas9-mediated knockout embryonic stem cells. Thus, we have generated both functional and descriptive multidimensional epigenomic dataset characterising the primary events during XCI. This investigation provides detailed roadmap of epigenetic events during rapid transcriptional silencing and provides insight into their function in developmentally programmed gene regulation.

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F-1176

ZFP371-DEFICIENT VERTEBRATE CELLS ACCUMULATE CHROMOSOMAL GAPS AND ACCELERATES CARCINOGENESIS IN MICE

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Zygotic genome activation (ZGA) begins after fertilization and leads to the totipotent state in preimplantation embryos. Genes specifically expressed at the ZGA stages would be expected to have important roles in establishing pluripotency and/or preimplantation development. Zspi (zinc finger and SCAN domain containing preimplantation gene) expressed specifically in preimplantation embryos, testis and embryonic stem cells (ESCs). ZSPI protein localized both in nuclei and cytoplasm at preimplantation stages and embryonic stem cells (ESCs). To characterize in vivo functions of Zspi, we generated a mutant mouse line with a targeted disruption of Zspi gene. Homozygous mice were then intercrossed to produce second-generation homozygotes, indicating that Zspi is not essential for fertility. Litter sizes of the intercrossed homozygotes were not significantly lower than those of wild-type (Zspi+/+) mice. On the contrary, surprisingly, teratomas formed from embryo-derived Zspi-/- ESCs were immature germ cell tumors with multilineage differentiated cell types. To reveal the cause of tumorgenesis, karyotype analysis using ESCs and spleen cells of Zspi deficient adults was performed. Although there were not significant chromosome abnormalities in ESCs, somatic cells exhibited chromosome instability in which chromosome gap, derivative chromosomes, gain or loss of chromosome, and robertsonian translocation were observed. Especially the chromosome gap frequency in Zspi-/- spleen cells was significantly higher than that of Zspi+/+ spleen cells (43.0% vs 1.3%, P=0.0010), and the location of gap occurred randomly throughout all of the chromosomes except chromosome 11. Zspi is reported to correlate with Rad51 and Brca1, both are onco-suppressor genes, shown by RNA-chromatin immunoprecipitation (RNA-ChIP). Our microarray data also showed elevated expression of Rad51l3 and Bard1 in Zspi deficient ESCs. These results imply that Zspi involve in DNA replication and/or DNA postreplication repair during mitosis. This is the first report that ZGA gene, Zspi, plays an important role in genome stability and preventing carcinogenesis.

F-1178

GENETIC INSTABILITY IN HUMAN EMBRYONIC STEM CELLS ASSOCIATED WITH SPECIFIC CULTURE CONDITIONS

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The application of human embryonic stem cells (hESCs) to provide differentiated cells for regenerative medicine require the successive and stable maintenance of the undifferentiated stem cells in prolonged culture. However, chromosomal stability during expanded passaging cannot be assured, as recent cytogenetic studies of human pluripotent stem cells (hPSCs) have shown recurrent karyotype abnormalities, including aneuploidies of chromosome 8, 12, 17, 20 and X. These chromosomal imbalance in hPSCs is a concern for clinical use and emphasizes the need for frequent assessment of karyotype. We have performed karyotype analysis in 375 cases of all 28 hESC lines cultured in conventional (STO feeder+20%KSR), human feeder (human foreskin feeder+20%KSR or 15%KSRxenofree) and feeder-free (mTeSR1+matrigel) culture system from 2006 to 2015. Karyotypic normal is 253/375 (67.5%) and abnormal is 122/375 (32.5%). In 193 cases of conventional culture, 149 cases were normal and 44 cases were abnormal. In 154 cases of human feeder culture system, 95 cases were normal and 59 cases were abnormal. In 28 cases of feeder-free system, 9 cases were normal and 19 cases were abnormal. All of 122 abnormal, trisomy 12 (34/122) and chromosome 20q isochromosome (34/122) were the predominant abnormalities. These karyotypic abnormalities were intensively analyzed by FISH and array CGH. This study are suggested that the genetic stability of hESCs are affected by various extracellular environments such as feeder cells, mediums, subculture techniques and extracellular matrices. Therefore, it is important to not only maintain optimal conditions for in

vitro cultures, but also to retain normal chromosome by karyotyping periodically for genetic stability in hESCs.

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F-1180

EPIGENETIC CONTROL OF HOX GENE EXPRESSION DURING CELL DIFFERENTIATION

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Correct Hox gene expression is critical for providing cells with an appropriate positional identity during embryonic development. Thus, the ability to understand and control Hox gene expression is crucial for the clinical application of in vitro cell differentiation protocols. In mammals, Hox genes are organized into four clusters which contain a subset of the 13 paralogous Hox genes. Individual Hox genes within a cluster are expressed in a position along the rostro-caudal axis that aligns with their physical location on the chromosome. Hox genes specify different spinal motor neuron subtypes at rostro-caudal positions along the spinal cord which dictate motor neuron connections required for voluntary muscle contraction. Hox gene expression is controlled by graded retinoic acid and fibroblast growth factors during neural tube development. Rapidly after receiving the rostro-caudal signal, Hox clusters are partitioned into two chromatin domains that control gene expression. CTCF binding at Hox chromatin state transitions is responsible for establishing the precise boundary between these two domains during directed differentiation of embryonic stem cells into cervical motor neurons (specified by Hox5 expression). Deleting a CTCF motif at the boundary between Hox5 and Hox6 leads to a shift in the chromatin boundary and activation of posterior Hox6 genes. Hoxc6 activation results in the transcriptional upregulation of Foxp1 and Raldh2, transcription factors that specify limb-innervating motor neuron identity at brachial levels of the spinal cord, as well as an increase in the proportion of Foxp1 positive motor neurons. Overexpression of Hoxc6 in a wild type genetic background induces the same homeotic transformation-like phenotype in differentiating stem cells. This indicates that the cell fate is transformed into a more caudal identity in the absence of CTCF-delimited chromatin boundaries. Therefore, patterning signals applied early during differentiation are epigenetically inherited through the cell lineage and control postmitotic motor neuron subtypes. Understanding how to control these stable chromatin boundaries will improve methodologies to regulate motor neuron fates and other Hox-dependent cell fates for clinical applications.

F-1182

DISSECTING THE TRANSCRIPTOME AND EPIGENOME SWITCH DURING HEPATIC LINEAGE CONVERSION

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The comprehensive understanding in induced pluripotency (iPS reprogramming) has rekindled an interest in understanding the direct conversion of the specialized cells from one lineage to another. Lineage conversion has become a powerful tool to study cell fate change, but limited studies have been made for understanding the dynamic changes during the early lineage conversion process. Here, we dissect the dynamics of transcriptome, histone modifications, chromatin accessibility and binding of key transcription factors during the direct hepatic lineage conversion. We find that gene expression start to change early from the intermediate stages. Active histone markers H3K27ac and H3K4me1 switch from early times positively correlated to early-stage gene expression changes, while repressive histone marker H3K27me3 shows dynamic change only at late stage, which may contribute to up-regulation of the late-induced hepatic genes. The early-induced genes are accessible for Foxa3 binding at early time, while the late-induced hepatic genes are located at repressive chromatin regions enriched by H3K27me3 modification, which indicates that H3K27me3 may be the barrier for hepatic lineage conversion. In addition, we find that Foxa3 prefers to binding to enhancer regions to regulate late-induced genes whereas it is more enriched at promoter regions of early-induced genes. These data shed light on how the hepatic features are established by forced expression of lineage specific transcription factors. We would also compare the direction lineage conversion and reprogramming during the meeting.

ORGANOIDS

F-1184

MIDBRAIN-LIKE ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS FOR DISEASE MODELING

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Parkinson's disease (PD) is caused by the selective and progressive loss of midbrain dopaminergic (mDA) neurons particularly from the substantia nigra pars compacta (SNpc). Due to restricted access to functional human brain tissue, human pluripotent stem cells (hPSCs) have been spotlighted as great cell source to generate mDA neurons. Indeed recent successful establishment of derivation methods have opened up novel opportunities for modeling PD in vitro. However, these 2D methods unlikely reflect the developing 3D nervous system and are inadequate to recapitulate the complexity and functionality of in vivo neural circuits. Very recently, whole brain organoids using hPSCs have been successfully generated by 3D culture system, which better mimic the tissue architecture and cellular interactions. In this study, we developed a method to differentiate hPSCs into human midbrain-like organoids (hMLOs) containing mDA neurons. First, embryoid bodies (EBs) were induced towards a floor plate fate via dual-SMAD inhibitors, Wnt pathway activator, and midbrain patterning factors. Subsequently, neural spheroids were embedded in Matrigel and transferred to tissue culture plates placed on an orbital shaker in organoid maturation media. These organoids grew to more than 2 mm in diameter in 1 month and contained multiple developing midbrain neuroepithelia. Global transcriptional profiling indicated a close resemblance to prenatal human midbrain. TH+ neurons in the hMLOs are electrophysiologically active, and produced dopamine. Strikingly, we detected neuromelanin (NM)-like granules, dark and insoluble pigment, in 3D hMLOs but not in 2D cultures of human mDA neurons. Single cell gene expression analysis of the NM-containing neurons revealed that these cells preferentially expressed markers of SNpc which are characterized as A9 subtype mDA neurons. Collectively, the hMLOs represent a potentially useful tool for in vitro modeling of human midbrain and its related disorders such as PD, and could serve as a versatile platform to understand pathophysiological mechanisms of PD.

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F-1186

MICRORNAS (MIRS) EXPRESSION DURING KIDNEY ORGANOID DEVELOPMENT FROM PLURIPOTENT STEM CELLS

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The kindey is a complex organ composed of more than 20 highly specialized cell types, which are responsible for filtration, fluid balance, and regulation of pH. 3D structures such as organoids are a functionally suitable ex vivo model for genetic disease studies and drug screening assays. Renal organoids have been derived from pluripotent stem cells, PSC. However, the molecular pathways that drive PSCs towards specialized renal organoids are a matter of current investigations. miRs are known to modulate multiple gene expression simultaneously, thereby regulating several signaling pathways. Many miRs have been described to be directly associated to the maintenance of PSC, while others are highly expressed in PSC undergoing differentiation, that makes them promising candidates for composing differentiation protocols. However, the roles of miRs during renal organoid development are still not fully revealed. In the present work, we focus on the study of miR expression during renal organoid development from different sources of PSC. Therefore, we first performed an in silico analysis aiming to define candidate miRs, whose expression levels are highly increased at the beginning of PSC differentiation. Next we reproduced 2 previously published differentiation protocols for renal organoid generation using 2 PSCs: ESC-H9 and CD34+-derived iPSC. Cells were harvested at distinct time-points for RNA extraction followed by reverse transcription of miRs and mRNAs. Untreated PSCs were used as controls. Various potentially interesting miR candidates as well as pluripotency-related genes and renal markers were analyzed by qRT-PCR. We confirmed the upregulation of metanephric markers, such as Six2, Pax2, and Osr1, as well as downregulation of Oct4 and Nanog, for both iPSC and H9-derived

organoids. Next, we show that a group of selected miRs was upregulated during organoid formation, compared to the PSC control. Intriguingly, these miRs target components of Wnt and pTEN-AKT signaling pathways, all of which are highly involved in PSC differentiation. Our findings contribute to the understanding of miR expression during differentiation of PSCs towards renal organoids as well as signaling pathways involved in the process. This knowledge will help us to optimize current protocols for kidney organoid generation from PSC.

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F-1188

USING NGLY1-DEFICIENT HUMAN PLURIPOTENT STEM CELLS TO UNDERSTAND CONGENITAL DEGLYCOSYLATION DISORDER-INDUCED NEURAL ABNORMALITIES

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NGLY1 gene mutations, leading to NGLY1 deficiency and associated with multiple neurological symptoms in pediatric patients, have been recently identified as the cause of a congenital deglycosylation disorder. However, how NGLY1 deficiency disturbs normal cerebral development and causes neurological abnormalities remains unknown. We have obtained NGLY1-deficient hESC by genome editing and generated patient-derived hiPSCs. To investigate the effects of NGLY1 deficiency on the early neural development, we have optimized two-dimensional and three-dimensional differentiation protocols for neurogenesis in the hESCs and hiPSCs. Loss of NGLY1 appears to have a negligible impact on the viability and cellular pluripotency in undifferentiated human pluripotent stem cells (hPSCs). Neuroepithelial spheres can be obtained in both control and NGLY1-deficient hPSCs, suggesting that the early-stage commitment of hPSCs to the neural lineage is not profoundly hindered by the loss of NGLY1 activity. Compared with the differentiated derivatives of control hPSCs, the neural derivatives of NGLY1-deficient hPSCs showed noticeably increased apoptosis, suggesting that NGLY1 activity may be critical for the viability of neural progenitor cells and the success of their subsequent differentiation. Using a high-content imaging approach, we are characterizing the cellular and biomarker features of control and NGLY1-deficient hPSCs that undergo two-dimensional neural differentiation after the neuroepithelial spheres are plated on a poly-ornithine/laminin-coated culture surface. Our findings indicated that NGLY1 deficiency causes a propensity of neural progenitor pool depletion and biases the cell fate decision during the differentiation of neural cells. We expect that our high-content imaging analysis in the neural derivatives of NGLY1-deficient hPSCs will shed lights on unprecedented information regarding the pathological development in patients with a recently-identified congenital disorder.

F-1190

HEPATOCELLULAR CARCINOMA EX VIVO MODELING AND DRUG SCREENING USING REPROGRAMMED HEPATOCYTES-DERIVED ORGANOIDS

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Hepatocellular carcinoma (HCC) is the one of the most common malignancies and common cause of cancer-related mortality worldwide. Sorafenib is the only approved targeted drug for advanced HCC. It is urgently needed to develop new drugs for this dreadful disease. In vitro techniques have not not reported to mimic the HCC development for drug screening and to translate data from the large-scale sequencing in HCC into specific therapeutic decisions. Organoids provide a good ex vivo model for HCC studies as it recapitulates in vivo microenvironment of solid tumor. We have established HCC organoid models by introducing specific mutations into reprogrammed hepatocytes (hiHep)-derived organoids. hiHep cells are functional hepatocyte-like cells derived from transdifferentiation of fibroblasts. We first introduced TP53 mutation and mutated CTNNB1 into the organoids, as these are two prevalent mutations affect 25%-30% of HCC patients. Comprehensively transcriptional and proteomic analyses of HCC organoids revealed genes and pathways with disease-stage-specific alterations. We also found that genetically engineered organoids express specific cancer markers (HSP70,GS, GPC3) and form cancerous structure ex vivo. Orthotopically transplanted these neoplastic hiHep organoids recapitulate the full spectrum features of HCCs. Genetically manipulation to hiHep organoids with hot spot mutations in HCC will help to efficiently screen targeted drugs. Besides these prevalent mutations, we also generated specific HCC organoid models with low-frequency mutation NFE2L2 to discover their functions in the cancer development which have not be achieved in the 2D cell lines culture system. Moreover, by sequentially introduce mutations into the organoids, we are able to model HCC development and test different hypothesis involved in HCC development. To sum up,

we have established ex vivo organoid model system for HCC studies.

F-1192

STEMDIFFTM HUMAN CEREBRAL ORGANOID CULTURE KIT: A NEW TOOL FOR THE CULTURE OF 3-D BRAIN ORGANOIDS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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2-D neural cultures derived from human pluripotent stem cells (hPSCs) are useful models to study the nervous system, but they are limited in their capacity to fully recapitulate the complex organization of brain tissues. Lancaster et al. (Nature 2013, Nature Methods 2014) have established a hPSC-based organoid culture system that models the major features of early human brain development. Based on these published media formulations, we have developed the STEMdiff™ Human Cerebral Organoid Culture Kit to enable generation of organoids in a simple and highly reproducible manner. The kit contains two basal media and four supplements, which are combined separately to prepare four complete media for each of the 4 stages of organoid formation. In stage 1, Human embryonic and induced pluripotent stem cells (hESCs or hiPSCs) maintained in mTeSR1™ were dissociated into single-cell suspensions and cultured in Organoid Embryoid Body (EB) Formation Medium (day 1-5). The EBs were then neuralized in the Organoid Neural Induction Medium (day 6 - 8, Stage 2); next, they were expanded by embedding in Corning® Matrigel® and grown in Organoid Neuroepithelium Expansion Medium (days 9 - 11 days, Stage 3). The expanded organoids were then cultured in the Organoid Maturation Medium, with agitation, for extended periods of time (day 12 - 40+, Stage 4). The organoids were collected at days 5, 8, 11 and 40 (Stages 1 - 4) and analyzed by RT-qPCR or cryosectioned for immunohistochemistry (3 organoids per analysis; 1 hESC, n = 2 and 1 iPSC, n = 2). A gradual increase in the expression of neuronal markers, beta III Tubulin, ASCL1 and TBR1, was observed as the organoids developed from stages 1 - 4. At day 40, organoids contained regions of PAX6+SOX2+Ki-67+ neural progenitor cells at the apical region, reminiscent of the ventricular zone (VZ). A separate population of dividing progenitors expressing Ki-67+ and p-Vimentin+ were detected adjacent to the VZ; this cell layer is similar to the outer subventricular zone found in primate cortex. TBR2+ intermediate progenitors were observed abutting CTIP2+MAP2+TBR1+ neurons, which resembled the intermediate zone and cortical plate. Taken together, our data are consistent with the published findings and demonstrate that the STEMdiff™ Human Cerebral Organoid Culture Kit supports the generation of cerebral organoids.

F-1194

OPTIMIZING THE DEVELOPMENT AND CHARACTERIZATION OF CANINE SMALL INTESTINE CRYPT ENTEROIDS AS A RESEARCH MODEL

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Orthotopic transplantation of intestinal organoids has the potential to repair or replace damaged epithelial tissues associated with chronic gastrointestinal disorders. However, progress in transplantation of intestinal organoids has been hampered due to the limited size and lifespan of mice. There remains, therefore, a critical need to develop well-characterized cell lines and protocols for cultivation and investigation of organoid delivery, efficacy and long-term safety following transplantation in large animal models. Dogs are more similar to people, when compared to mice, in many aspects including genomic makeup, anatomy/physiology, and susceptibility to infectious disease. This is the first report of successful propagation of canine intestinal organoids ex vivo. Ten centimeter pieces of proximal jejunum were acquired from 4 young healthy dogs, washed in PBS and minced. Intestinal crypts were enriched, using EDTA chelation, released via trituration, embedded in matrigel, and grown in intestinal stem cell media. Optimal factors for canine organoid isolation and culture including rho-associated kinase ROCK inhibitor Y27632, glycogen synthase kinase 3β inhibitor CHIR99021 and wnt-3a were evaluated to maximize growth performance. Aliquots of organoids were cryopreserved in liquid nitrogen for future analysis. Preliminary morphological characterization included live cell brightfield imaging, Trypan Blue viability staining and H&E. Canine organoid culture has been successfully carried greater than 8 passages. Preliminary results suggest that the addition of 50 ng/mL of rho-associated kinase inhibitor produced more rapid growth and larger organoids over three generations. Morphological

characteristics apparent from H&E include proliferation via relatively abundant mitotic figures, classic ordered simple columnar epithelial polarity with basal nuclei and clear cytoplasm of goblet cells.

TISSUE ENGINEERING

F-1198

GENETICALLY ENGINEERED CARDIAC FIBROBLASTS FOR ELECTRICAL MATURATION OF HIPSC-CARDIOMYOCYTES IN ENGINEERED TISSUES

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Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) are an invaluable therapeutic resource for myocardial repair, however, their functional immaturity remains an obstacle in clinical application. Regarding electrical maturity, hiPSC-CMs have lower expression levels of the gap junction protein connexin 43 (Cx43), decreased conduction velocity of action potentials, and decreased maximum capture rate than adult cardiomyocytes. One approach to address this inherent immaturity is to include supporting cell types in engineered cardiac tissues, which recapitulates in vivo cellular composition. We employed this method to test the hypothesis that human cardiac fibroblasts (hCFs) with lentiviral upregulation of Cx43 expression would improve structural integrity and electrical functionality of engineered cardiac tissues. Engineered tissues were formed with 1x106 11-14 day-old hiPSC (Gibco episomal hiPSC line)-CMs in 1.25 mg/mL rat tail collagen-1. To determine optimal hCF content, primary adult hCFs (PromoCell) were doped into engineered cardiac tissues at 5, 10, and 15% of input hiPSC-CM number. HCFs significantly increased tissue compaction by up to 1.5-fold after 5 days in culture ($P < 0.05$). HCFs also increased maximum capture rate of tissues in a dose-dependent manner ($P < 0.05$). HCFs infected with lentiviral expression of GJA1, encoding Cx43, upregulated viral gene expression as determined by a GFP reporter and expression of membrane-localized Cx43 by immunofluorescence. Q-RT-PCR analysis confirmed that GJA1 expressing hCFs expressed mRNA levels of GJA1 not significantly different than GFP-control hCFs or hiPSC-CMs. Optical mapping of membrane voltage using RH237 in engineered cardiac tissues containing hiPSC-CMs and 5, 10, or 15% control hCFs suggest a dose-dependent slowing of conduction velocity with increasing hCF numbers from 0.06 to 0.04 mm/ms. Ongoing studies using Cx43-overexpressing hCFs aim to modulate tissue-level conduction velocity through heterotypic cellular interactions and increased fibroblast electrotonic potential. This engineered tissue platform enables advancement of stem cell-based therapies towards heart regeneration and interrogation of the interactions between hiPSC-CMs and hCFs.

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F-1200

FRACTIONAL CO2 LASER INDUCES A QUORUM SENSING LIKE EFFECIENT REGENERATION

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It is not surprising that injury can induce a regeneration response. Generally, the regeneration process is thought to occur via a one-to-one condition whereby stimulation of one stem cell leads to the activation of that single stem cell. However, this is an inefficient regeneration process. Recently, we accidentally discovered that regeneration could occur through a collective decision making process. By plucking hairs with a proper arrangement, up to 5 times more neighboring, unplucked resting hairs were activated to regenerate. However, if the number of plucked hairs was below a threshold, no hairs including the plucked ones regenerated. This type of regeneration is a threshold dependent all-or-none process, which provides an organ-level example of quorum sensing. Quorum sensing is a self-driven decision-making process when certain criteria are met within the responding population. In recent years, fractional CO2 laser was used to treat scar and rejuvenation. Fractionation is the concept that we don't ablate the whole skin but deliver energy into myriad microbeams. Through control the density and energy we deliver, we can let the patient lease only very tiny spot injuries without downtime. The way how fractional CO2 laser performs is just similar to our recent proportional hair plucking. According to this, we hypothesize that this device can induce a "quorum sensing" like hair regeneration. In this study, we perform different densities and energies of fractional CO2 laser on the back skin of mice and find out that only when proper energy and density of CO2 fractional laser was delivered, the regeneration can occurred. If the emission density is too low or the energy is not strong enough, no regeneration can happen. Interestingly, when hair grow happened, all the area receiving the energy will simultaneous regenerated, just like the "quorum sensing" behavior we published before. Analyzing the molecular bases, we identified that immune response as well as growth factor activation, including KGF and HGF are involved in this process. According to this, we think fractional CO2 laser can induce an efficient hair

regeneration process. By utilizing this device, we think we can help the patients with hair loss in the future.

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F-1202

ATTEMPT TO GENERATE THE TARGET GENE KNOCK-IN/KNOCK-OUT MARMOSET USING CRISPR/CAS9

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The common marmoset (marmoset, Callithrix jacchus) is a non-human primate, that can be used to generate genetically modified (GM) models. GM marmosets would be powerful experimental animals for assessing the safety and efficacy in regenerative medicine and new drug. Genome-editing technologies make it possible to produce marmosets with modified target genes. Last year, we reported genome editing in the marmoset using zinc-finger nucleases and transcription activator-like effector nucleases (TALEN). However, the newest genome-editing technology, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas), has not been well studied for genome editing in marmoset embryos. The c-kit proto-oncogene encodes Kit tyrosine kinase (Kit), which is a receptor for stem cell factor. In humans and mice, Kit plays important roles in hematogenesis, gametogenesis and melanogenesis. Therefore, a c-kit knock-out marmoset would be very useful model for investigating the molecular mechanisms of hematogenesis, gametogenesis and melanogenesis in non-human primates. This study, investigated the most efficient conditions for CRISPR/Cas9 that targeted to the marmoset c-kit gene in marmoset embryos. In the first study, the optimal concentration of single-guide RNA (sgRNA) and Cas9 mRNA were examined. The concentration of 5 to 100 ng/μL of sgRNA and 20 to 180 ng/μL of Cas9 mRNA were injected into marmoset embryos. As the result, the combination of 50ng/μL sgRNA and 100 ng/μL Cas9 mRNA had the highest genome-modifying efficiency. In addition, two kinds of humanized Cas9 mRNA, Church's Cas9 and Huang's Cas9, were also compared. Huang's humanized Cas9 mRNAs exhibited better cleavage activity (78.9%) and better embryo development. Furthermore, Cas9 nuclease was also injected into marmoset embryos to compare the efficacy of genome editing of Huang's Cas9 mRNA. The results showed that Cas9 nuclease had the highest efficiency of modification (80%) of the marmoset embryo genome. This condition also applied when modifying the marmoset Shank3 gene, which is responsible for the development of idiopathic autism spectrum disorders. CRISPR/Cas9 would be one of effective method for producing marmoset models of human diseases for study of regenerative medicine by target gene knock-out and knock-in.

F-1204

MICROFLUIDIC TISSUE ENGINEERING OF PERFUSABLE 3D VASCULAR NETWORKS ON A CHIP USING HUMAN IPS CELLS

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Engineering of functional vascular networks is an important goal for tissue engineering as well as for study of vascular biology. To date, several 3D perfusable microvascular networks have been developed in vitro; however, these models have some limitations because most of current models were developed with primary human endothelial cells (ECs) such as human umbilical vein endothelial cells (HUVECs). To investigate the interaction of blood vessels with circulating cells such as cytotoxic T cells, allo reaction between HUVECs and T cells by mismatching of their HLA will be problematic. Here we demonstrate a perfusable blood vessel model engineered by human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) using the microfluidic-based tissue engineering platform. The hiPSC-ECs were produced by the rapid and robust differentiation protocol towards mesodermal fate, including GSK3β inhibition and BMP4 treatment. The hiPSC-ECs characterized by the expression of typical endothelial markers (CD31 and CD144) showed a similar angiogenic activity to HUVECs in the tube formation assay using growth factor-reduced Matrigel. We found that the ratio between venous and arterial vascular endothelial progenitors (segregate to the CD34+CD73+CD184- and CD34+CD73+CD184+ fractions, respectively) derived from hiPSCs can be controlled by the surface treatment. The fibronectin-coated surface increased venous progenitors. To engineer functional vascular networks, the hiPSC-ECs were cultured in a fibrin gel and induced angiogenesis by co-culturing with human lung fibroblasts (LFs) in a microfluidic device. We confirmed that the co-culture of LFs induced the formation of 3D vascular networks which can be perfused with culture medium as well as blood cells. Generation of in vivo-like perfuseble 3D vascular networks with these characterized hiPSC-ECs would be beneficial to better understand the function of venous and arterial vascular endothelial progenitors

and provide a platform to develop vascularized organ-on-a-chip systems and human disease models for pharmaceutical screening.

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F-1206

REGENERATION OF RAT TRACHEA USING DECELLULARIZED TRACHEA SCAFFOLD AND MOUSE IPS CELL FOR IN VIVO APPLICATION

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Tracheal transplantation for the treatment of airway stenosis due to congenital airway stenosis, malignant tumor invasion, chronic inflammation and scar has been confronted with several unsatisfactory outcomes. We investigated the feasibility of regeneration of trachea using rat decellularized trachea scaffold and mouse iPS cell for in vivo application. A rat trachea was firstly decellularized using a detergent-enzymatic treatment method previously reported. We successfully established an original centrifugal method to transplant cells onto the luminal surface of the decellularized rat trachea scaffold circumferentially. Mouse iPS cells were differentiated into definitive endoderm cells and transplanted onto the luminal surface of the decellularized tracheal matrix scaffold using this centrifugal method. Then F344/NJc1-rnu/rnu rats were anesthetized and five rings tracheae were removed and orthotopic tracheal transplantations were carried out with recellularized trachea scaffolds. The nud rats transplanted with recellularized trachea scaffold survived 2-4 weeks. Histologic analysis indicated the cause of death was the airway stenosis due to the colonic cellular proliferation of undifferentiated iPS cells. The nud rats transplanted with no cell scaffold survived over one month, though the airway stenosis due to the deficiency of tracheal cartilage was also observed. We will next try to investigate the possibility of recovering airway stenosis by transplanting mouse primary tracheal epithelial cells onto the luminal surface of trachea scaffold using the centrifugal method and transplanting the chondrocytes differentiated from mouse iPS onto the outer membrane of the trachea scaffold using cell sheet engineering.

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F-1208

TRANSGENIC EXPRESSION OF EPHB4 IN STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH PROMOTES OSTEOGENIC CAPABILITY

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The aim of the present study was to evaluate the effects of stem cells from human exfoliated deciduous teeth (SHED) that transfected with EphB4 gene in regulating osteogenic differentiation. Human dental pulp tissue were harvested from extracted deciduous teeth and digested by Collagenase and Dispase. The SHEDs were transfected with transgenic (hEphB4-GFP) vector or empty vector (GFP-vector). Real-time PCR analysis and western blot were used to detect the expression of EphB4 in SHEDs after transfection. EphB4-SHEDs and GFP-SHEDs were subjected to osteogenic induction and assessed by alkaline phosphatase (ALP) assay and Alizarin-red Staining. Real time-PCR revealed that the expression of EphB4 m-RNA in EphB4-SHEDs was significantly higher than that of GFP-SHEDs ($P < 0.05$). The expression of EphB4 protein in EphB4-SHEDs was also significant higher ($P < 0.05$) than that of GFP-SHEDs. ALP assay and Alizarin-red Staining demonstrated higher ALP activity and increased mineralization with EphB4-SHEDs. Our data indicated that transgenic expression of EphB4 in SHEDs promotes osteogenic differentiation.

F-1210

ENHANCEMENT OF DENTAL PULP REGENERATION BY THE COMBINATION OF DPSCS TRANSFECTED WITH VEGF AND SDF-1A

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Dental pulp stem cells (DPSCs), a subpopulation of pulpal cells, have the potential to proliferate and differentiate into odontogenic lineage during repair and regeneration processes. Pulp tissue could be regenerated by combining DPSCs, scaffold and growth factors if a sufficient blood supply into the root canals is secured. In this process, vascularization is vital for the survival of cells after implantation in pulp regeneration in vivo. However, it is not clear how the angiogenic factors, VEGF and/or SDF-1α act on regenerative potential in DPSCs. In this study, the vascularization potential of DPSCs transfected with VEGF or SDF-1α was investigated in vitro and in vivo. After gene modification, RT-PCR, ELISA and western blot analysis verified stable gene and protein overexpression. Transfected DPSCs have higher cell proliferation and

enhance HUVECs migration and tube formation in vitro. The root segments mixed with gene-modified DPSCs and PuraMatrix were implanted in severe combined immunodeficient (SCID) mice and retrieved after four weeks. The histology and immunohistochemistry results demonstrated that regenerated pulp could be achieved by transplantation of DPSCs and PuraMatrix in vivo. The combination of DPSCs/VEGF and DPSCs/SDF-1α generated significantly more volume of pulp-like tissues than DPSCs/VEGF, DPSCs/SDF-1α or wild-type DPSCs group

F-1212

IN VIVO ANGIOGENIC CAPACITY OF DECELLULARIZED DIAPHRAGM

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The aim of skeletal muscle tissue engineering is to provide a suitable graft to support and repair large congenital and acquired defects. Successful engraftment after transplantation depends on immunomodulation, to avoid rejection, and vascularization, to guarantee cell survival, growth and overall regeneration. In the past decade, biologically-derived decellularized scaffolds have been described as a good tool for tissue engineering purposes since they possess the intrinsic characteristics to maintain both the architecture and the composition of the original tissue. Decellularized scaffolds are mainly composed of extracellular matrix, a unique combination of proteins and cytokines that synthetic scaffolds do not possess. Our group recently developed a diaphragm-derived decellularized scaffold, which after implantation led to a regenerative immunological response and to the improvement of an atrophic diaphragm performance. In this work, we focused on the ability of our acellular scaffold to promote vascularization. Angiogenic and vasculogenic potentials of commercially available polymer and decellularized diaphragm have been compared both in vitro using Chicken chorioallantoic membrane (CAM) and in vivo after back skin and orthotopic implantation. Proteome profiler angiogenesis array, ELISA tests, Haemoglobin quantification, immunofluorescence and qReal Time-PCR have been settled to analyze the attained results. The CAM assay and protein array confirmed the presence of pro-angiogenetic molecules in the decellularized tissue. After transplantation, scaffold was remodelled and formation of functional vessels was detected after 7 and 15 days. From the comparison with a synthetic material currently in clinical use we could confirm the high potential of a diaphragm derived acellular matrix as a scaffold for skeletal muscle tissue engineering purposes.

ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

F-1218

STEM CELL SCIENCE PUBLIC POLICY IN THE WAR AGAINST ZIKA

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Reports about the re-emergence of Zika virus in South America during the 2015/2016 Southern Hemisphere summer and the possible correlation between Zika virus infection and neuronal pathologies in infants born to infected mothers, as well as adults, rocked the world and led to the declaration of a global health emergency by the World Health Organization. Within what many regard as an extraordinarily short period of time, collaborations of virologists, epidemiologists, and stem cell scientists developed models of these pathologies using human pluripotent stem cell-derived neural progenitors and brain organoids, fetal tissues, and animal models. From these models, various hypotheses quickly came to the fore about the cellular components targeted by the virus, the mechanisms of action employed by the virus, and the pathogenetic impact of the infection. Beyond these hypotheses, these models have provided platforms for testing medical countermeasures against the virus. From a public policy perspective, the speed and impact of these collaborations raise at least two questions: (1) what role did public policy play in fostering these collaborations and their output? and (2) how can lessons about this role of public policy be applied to improvements in those policies and their widespread implementation around the globe?

F-1220

CLINICAL APPLICATION OF PLURIPOTENT STEM CELLS AND ITS FUTURE

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Since the establishment of human embryonic stem cells (hESCs) in 1998, several attempts have been made to use hESCs for clinical application. In 2010, Geron Corporation initiated the first phase I clinical trial of hESC-based therapy for spinal cord injury though it was eventually cancelled after 4 cases of transplantation. This was followed by trials aiming to use hESCs to treat age-related macular degeneration in the US and China, a trial for heart failure in France, as well as type I diabetes in the US. While the science of hESCs and their clinical applications has been developing, the field has been challenged by shifting political and legal winds. Due in part to religious objections to the requirement for embryo destruction, the Bush Administration tightened federal funding to hESC research in 2001, but this restriction was later lifted by the Obama Administration lifted in 2009. In the EU, the Court of Justice concluded in 2011that hESC or hESC-derived biomaterials are not patentable, but this was followed by a decision in 2014 that hESC and derivatives can be patentable if hESCs are established from unfertilized eggs. A potential resolution to some of these controversies has come from human induced pluripotent stem cell (hiPSC), which are developed without embryos. With this understanding, governments including in Japan have increased research funding for hiPSC research since 2007 when the establishment of hiPSC were first reported. This funding resulted in the world's first retinal replacement surgery using hiPSC, which was successfully performed in September 2014 in Japan. The safety concern of hiPSC, however, has limited their application as can be seen from the example that the research team could not conduct second and later surgeries due to the discovery of a mutation in the hiPSC line used, which was hard to be assessed. We review the political, legal, and ethical controversies affecting the current and future clinical application of pluripotent stem cell.

F-1222

REGULATING STEM CELL RESEARCH & TREATMENTS: CLOSING THE GAP

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Certain types of stem cell research are controversial and many stem cell treatments are currently unproven. Embryonic stem cell research is contentious as extraction of stem cells from a human embryo involves its destruction. Accordingly, in many nations, it is often recommended that stem cell research and therapy should be strictly regulated. However, in regulating new technologies, there are various challenges that regulators face. This presentation refers to the writings of Professor Roger Brownsword (Kings College London), a bioethicist and scholar on issues of technology, ethics and law. He acknowledges the need for the exploration of the social, legal, ethical and regulatory issues arising from the emergence of modern technologies. The main difficulties of regulating innovative technologies identified by him are as follows: the challenges of achieving regulatory legitimacy, attaining regulatory effectiveness and maintaining the regulatory connection. It is critical that regulators bear in mind the difficulties that they may encounter in regulating innovative technologies. Brownsword warns that unless these challenges are sufficiently addressed, the regulatory environment is defective as opposed to a regulatory framework that supports the development, application and exploitation of technologies that will contribute to an overarching purpose, an environment properly geared for risk management as well as benefit sharing. Therefore, to construct an effective regulatory framework for emerging technologies, the regulators must recognise the difficulties likely to be faced and attempt to design and sustain a regulatory environment that is perceived to be legitimate, effective and is connected to the changing technology. While Brownsword's work refers to regulatory challenges presented by innovative technologies generally, they are relevant in the context of the regulation of stem cell research and therapy. This presentation also considers measures that could be adopted by regulators to counter the difficulties with reference to Professor John Braithwaite's (Australia National University) responsive regulatory theory. Interviews were conducted with Professor Brownsword and Professor Braithwaite to seek their views on these issues and possible strategies on how to meet the challenges.

CLINICAL TRIALS AND REGENERATIVE MEDICINE INTERVENTIONS

F-1224

EX VIVO MESENCHYMAL STEM CELL THERAPY

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Human mesenchymal stem/stromal cells (MSCs) metabolize and secrete molecular mediators that can globally shift a wound healing response. Controlled exposure to this cell therapy has been challenging with intravenous infusion of MSCs due to limits in tolerable cell dose and the rapid clearance of MSCs by the body. We have developed an ex vivo MSC technology that maintains MSC viability and enables the continuous, controlled delivery of MSC molecules into the blood stream in a clinical setting. MSCs were integrated into hollow-fiber bioreactor devices whereby the cells, separated by a permeable membrane, can directly and dynamically condition a patient's blood without entering the body. A human scale prototype of the technology will be presented showing sustained cell viability and function throughout cGMP manufacturing. Pharmacological analysis of this bioreactor technology in a large animal toxicology study allowed for an unprecedented look at MSC therapy during product use. The study verified a pharmacokinetic and pharmacodynamic response to extracorporeal MSCs that is consistent with a potent immunomodulatory mechanism of action in large animals. The presentation will also report encouraging in vivo survival results of ex vivo blood conditioning with MSCs in a canine model of ischemic acute kidney injury (AKI). A Phase I clinical trial design and early enrollment in AKI patients will be announced as well. Ex vivo blood conditioning with this MSC reactor technology can find many other clinical applications in regenerative medicine and immunotherapy.

Funding Source: Funding provided by Sentien Biotechnologies, Inc.

F-1226

PHASE I CLINICAL TRIAL DEMONSTRATES SAFETY AND EFFICACY OF REPEATED INTRATHECAL MESENCHYMAL STEM CELL-DERIVED NEURAL PROGENITORS IN PROGRESSIVE MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic autoimmune disease with disseminated inflammatory central nervous system demyelination and axonal damage. In many patients, MS becomes progressive over time, leading to an accumulation of irreversible neurological disability. Therapies aimed at preventing or slowing progressive MS by enabling repair and neuroprotection are still lacking. We have investigated a neural progenitor-like subpopulation of mesenchymal stem cells (MSC-NPs) that exhibit immunoregulatory and trophic properties both in vitro and in vivo. The objective of this FDA-approved phase I, open label clinical trial was to determine safety and tolerability of autologous MSC-NPs administered intrathecally (IT) in 3 doses of up to 10 million cells per injection, spaced 3 months apart. Twenty MS patients with established disability and relatively stable disease were enrolled. MSC-NPs expanded from autologous bone marrow were batch-tested for quality, sterility, and chromosomal stability. Primary safety outcomes included adverse event assessments. Secondary outcomes to observe efficacy included change in muscle strength (MRC), EDSS (expanded disability status score), timed 25 foot walk (T25FW), and urodynamics testing for bladder function. Repeated dosing of MSC-NPs was safe and well tolerated, with only minor adverse events including transient headache and fever. Of the 20 study subjects, 15 (or 75%) demonstrated functional neurological improvement associated with IT-MSC-NP treatment. These positive trends in efficacy demonstrate functional neurological improvement within a group of MS patients who had otherwise stable disability for a year prior to the study. Improvements were more frequently observed in subjects who were ambulatory (EDSS $\leq$ 6.5) upon enrolling in the study. The encouraging safety and efficacy results from the phase I trial warrant a larger phase II placebo-controlled study to confirm efficacy of intrathecal MSC-NP treatment in patients with MS.

F-1228

DIRECTLY REPROGRAMMED HUMAN NEURAL PRECURSOR CELLS PROMOTE FUNCTIONAL RECOVERY IN A MOUSE MODEL OF SENSORIMOTOR STROKE

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Current treatment strategies for stroke offer limited success, making it one of the leading causes of acquired long-term disability worldwide. Cell transplantation is a promising therapeutic intervention. However, several hurdles, including the identification of an optimal cell type and source, still exist. Herein, we explore the efficacy of a novel population of directly reprogrammed human neural precursor cells (drNPCs) to treat the stroke-injured brain. Briefly, somatic cells were isolated and transformed via transient expression of Musashi-1 (Msi1), Neurogenin-2 (Ngn2), and Methyl-CpG Binding Domain Protein 2 (MBD2), generating drNPCs within 2 weeks of transfection. These cells afford the benefit of using patient specific somatic cells and directly reprogramming them to NPCs, without the use of viral constructs, thereby providing a safe and ethically sound source of autologous cells that can avoid immune rejection and bypass risks associated with pluripotency. Using immunohistochemistry and PCR, we confirmed drNPCs comprise a population of stem and progenitor cells committed to the neural lineage. To examine the potential of drNPCs to promote neural repair and functional recovery following ischemia, we established an endothelin-1 model of stroke in the sensorimotor cortex of immunocompromised SCID/Beige mice that results in long term motor deficits, which permits us to examine functional recovery and avoid immunorejection of drNPCs following transplantation. The drNPCs were transplanted into the lesion site 4 days post-stroke in artificial cerebrospinal fluid (aCSF) or a hyaluronan methylcellulose (HAMC) hydrogel that has been shown to promote cell survival. Stroke-injured mice that received aCSF alone, HAMC alone, drNPCs+aCSF, and drNPCs+HAMC were compared. Sensorimotor behavioural assays, immunostaining, and lesion volume outcomes were used to measure functional recovery, cell survival and differentiation, and tissue regeneration. We found that lesion volume was not significantly different in any treatment group 32 days post-stroke. Interestingly, at 32 days post-stroke we observed significant functional recovery in mice that received drNPCs. These findings reveal that drNPCs are a promising cell source for neuroregenerative strategies to treat the stroke-injured brain.

Funding Source: Canadian Institutes of Health Research, New World Laboratories Inc., Canada First Research Excellence Fund

F-1230

SECRETION PROFILE OF PARACRINE FACTORS PREDICTS PROANGIOGENIC EFFICACY OF MESENCHYMAL STROMAL/STEM CELLS

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Mesenchymal stromal/stem cells have been used in the clinic for cell therapy of ischemic disease. However, there are some hurdles in clinical application such as poor cell engraftment or inconsistent stem cell potency. Previously, we have reported that MSCs derived from Wharton's jelly (WJ-MSC) promote neovascularization by secreting paracrine factors and serving as pericyte-like cells. In this study, we investigated biomarker for predicting stem cell potency by carrying out comparative studies about the relative proangiogenic activities of WJ-MSCs derived from different donors. Proangiogenic factor secretion profiles of the conditioned media derived from various WJ-MSCs were varied from donor to donor, and 9 among 55 angiogenesis-related factors were secreted at the considerable levels. We further meta-analyzed the same proangiogenic factor set data from several MSCs derived from various sources, and found that these 9 factors were also highly but variably secreted. Interestingly, 2 distinct WJ-MSCs that show low or high secretion of proangiogenic factors showed corresponding proangiogenic activity in vivo in the Matrigel plug assay. We further validated the relationship between secretion profile and proangiogenic efficacy using 4 different donors of WJ-MSCs. As expected from the secretion profile, WJ-MSC from Donor D8 showed the most potent proangiogenic activities in proliferation, migration and tube formation of endothelial cells (ECs) in vitro, and also in in vivo Matrigel plug assay. In addition, D8 WJ-MSC-derived conditioned medium potently induced phosphorylation of FAK and ERK1/2 in ECs. From the data, 4 cytokines were finally chosen for minimal biomarkers for proangiogenic efficacy of MSCs. Accordingly, blocking of each cytokine by neutralizing antibodies significantly inhibited the angiogenic activities of WJ-MSCs. Therefore, these results suggest that secretion levels of 4 cytokines may represent the proangiogenic potency of WJ-MSCs and that these 4

cytokines set can be used as a prediction biomarker for effective proangiogenic cell/stem cell therapy.

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GERMLINE, EARLY EMBRYO AND TOTIPOTENCY

F-2002

INDUCTION OF TOTIPOTENT FRACTION IN ES CELL CULTURE

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In mammals, cells from very early-stage embryos have the ability to generate both embryonic and extra-embryonic cell types and thereby be defined as totipotent cells. In the mouse, the first specialization event occurs at around the 16-cell stage. At this stage, the polarized outer cells of the embryo begin to differentiate towards trophoblast while the inner cells start to differentiate to inner cell mass (ICM). Embryonic stem cells (ESCs) can be derived from ICM of blastocyst and maintain the capacity to make all the somatic lineages and the germ cells, but not the extra-embryonic lineages. Therefore, ESCs are thought to pluripotent cells, which lack the ability to make all extra-embryonic tissues. However, recent study revealed that a rare transient fraction within ESCs culture, that expresses high levels of murine endogenous retrovirus with leucine tRNA primer (MuERV-L). Importantly, MuERV-L expressing ESCs lack the pluripotency associated proteins Oct4, Sox2 and Nanog, and have acquired the ability to contribute to both embryonic and extra-embryonic tissues. In this study, we defined culture condition for ESCs that leads to increases the population of MuERV-L positive cells. We examined whether a single tdTomato positive cells have acquired the ability to differentiate to extra-embryonic lineages when injected into morula-stage embryos. We will discuss the gene expression and differentiation potential of a single MuERV-L positive cell.

F-2004

EPIGENETIC LANDSCAPE OF MOUSE GASTRULA

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Regionalized lineage progenitor cells emerged from the onset of gastrulation to facilitate the embryonic patterning and build a blueprint for future development. However, the molecular mechanism especially the epigenetic mechanism underlying the formation and development of lineage restricted progenitors cells remain unknown. Here, we analyzed the transcriptome, DNA methylome and histone modification landscape of region restricted lineage cells based on carefully embryo manipulation. We found that development related master transcription factors are tightly regulated by epigenetics. Different epigenetic mechanisms are involved to maintain transcriptome profile of region specific lineage cells. Large numbers of gastrula specific enhancers were recruited to promote the operation of gastrulation. Region specific super-enhancers are critical for the identity of lineage cells. Motif analysis also identified key regulators of lineage development during gastrulation. These data revealed a comprehensive epigenetic landscape for mouse gastrula and broadened our understanding of mammalian embryo development.

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PLURIPOTENCY

F-2006

BABOON AND HUMAN PLURIPOTENT STEM CELLS SHARE SIMILAR GENE EXPRESSION SIGNATURES

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A major hurdle in the clinical implementation of stem cell-based therapies is the lack of translatability of therapeutic approaches between typical model

organisms and human patients. Additionally, a significant practical concern is the need to rapidly assess the pluripotency and differentiation potential of individual pluripotent cell lines. To address both of these concerns, we evaluated human and baboon ES and iPS cells using a gene expression-based approach to evaluate the pluripotency and differentiation potential of individual cell lines. We found that baboon and human ES and iPS cells are similar in their expression levels of key pluripotency genes and that their gene expression profiles could be correlated to their propensities to differentiate into progenitors of endoderm, ectoderm, and mesoderm. These results not only confirm the utility of gene expression-based assays to predict function across different species, they also validate the baboon as an optimal translational model for stem cell therapies by highlighting the similarities between human and baboon pluripotent stem cells.

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F-2008

EFFECTS OF OXIDATIVE AND THERMAL STRESSES ON STRESS GRANULE FORMATION IN PLURIPOTENT STEM CELLS

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Stress Granules (SGs) are ribonucleoprotein aggregates or RNA granules, which had been previously observed in different types of cells subjected to environmental stresses such as hypoxia, oxidative stress, and heat shock. These granules are dynamic in nature and are induced by the phosphorylation of eukaryotic translation initiation factor 2 α (eIF2α), a key regulatory factor in translation initiation. Thus, these granules are considered a part of the stress response program that is known to play an essential role in regulating the translation process. It is known that pluripotent stem cells are highly sensitive to oxidative stress, indicating the importance of the stress response program in regulating stem cell fate. In this study we determined the effect of oxidative and thermal stresses on SG formation in pluripotent stem cells to eventually establish whether these granules play a role in regulating self-renewal and differentiation. To study SG formation in pluripotent stem cells, induced pluripotent stem cells (iPSCs; IMR-90) were treated with sodium arsenite (SA) and hydrogen peroxide (H2O2) (oxidative stresses) and heat shock (HS) (thermal stress), then cells were fixed, stained with the robust SG marker (G3BP), and quantified for SG formation using fluorescence microscopy. Our results showed that SA and HS, but not H2O2, induce SG formation in iPSCs. In SA treated cells, SGs were formed in dose dependent manner, where the number of cells displaying SGs progressively increases with the increase of SA concentration. However, iPSCs treated with H2O2 exhibited no SG formation even with higher concentrations or longer incubation periods. On the other hand, no granules were observed in cells kept at 37oC or exposed to mild HS (40oC) treatment, whereas at higher temperature of 42oC, 100% of the cells formed SGs. We also demonstrated that the well-known protein components of SGs (G3BP, TIAR, eIF4E, eIF4A, eIF3B, eIF4G, and PABP) were recruited to the granules formed in iPSCs treated with SA or subjected to HS. Consistent with the SG data, SA and HS, but not H2O2, promote eIF2α phosphorylation in iPSCs forming SGs. Altogether, these data demonstrate that not all stressors are capable of inducing SGs in iPSCs and that the granules formed in stressed iPSCs are typical SGs in terms of size, number, and types of protein recruited.

F-2010

DETERMINING THE ROLE OF CULLIN9-MEDIATED CELL CYCLE CONTROL IN HUMAN PLURIPOTENT STEM CELL SELF-RENEWAL AND DIFFERENTIATION

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While there are major differences between the cell cycle of pluripotent stem cells (PSCs) and somatic cells, investigation into the unique cell cycle regulatory mechanisms of PSCs has been limited. The overall length of the PSC cell cycle in humans and mice is shorter than that of somatic cells, and the G1 and G2 phases are abbreviated. As PSCs differentiate, the G1 and G2 phases gradually lengthen, and the cell cycle begins to resemble that of a somatic cell. While novel cell cycle control mechanisms have been identified in mouse PSCs (mPSCs), the elucidation of regulatory mechanisms in the human PSC (hPSC) cycle has been unsuccessful. Our preliminary data indicates that an E3 ubiquitin ligase known as Cullin-9 (Cul9) is involved in hPSC cell cycle control. E3 ubiquitin ligases are enzymes that modify targeted proteins with ubiquitin moieties, which can signal for proteasomal degradation, or alteration of the target's localization, function, or protein interactions. Despite Cul9's elevated expression in hPSCs, there are

currently no known Cul9 substrates in hPSCs. Through an unbiased approach by mass spectrometric analysis, we identified several subunits of the anaphase promoting complex/cyclosome (APC/C) as Cul9 interacting proteins in hPSCs. The APC/C is a multi-subunit E3 ubiquitin ligase that catalyzes the ubiquitin-mediated proteasomal degradation of key substrates involved in mitotic progression and maintenance of G1 phase. I will show our supporting preliminary data that Cul9 may regulate G1 length through modulation of the APC/C, thereby acting as a unique hPSC cell cycle checkpoint critical for self-renewal and differentiation.

F-2012

TRANSCRIPTIONAL ROLES OF RELA AND RELB NF-KB SUBUNITS IN PLURIPOTENCY MAINTENANCE AND DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS AND EMBRYONAL CARCINOMA NTERA-2 CELLS

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Transcription factors (TF) such as OCT4, NANOG, SOX2, LIN28, KLF4, and MYC are centrally involved in the maintenance of human pluripotent stem cells (hPSCs) identity, however, additional TFs and pathways are also involved in this complex regulatory network. There are conflicting results to whether the classical or the alternative non-canonical NF-kB pathways, mediated, by RELA(p65)/NFkB1(p50) or RELB/NFkB2(p52) TF complexes, respectively, would be involved in the regulation of pluripotency or differentiation. Previously, we showed that upon atRA-induced differentiation of the pluripotent embryonal carcinoma NTera-2 cell line, transcript levels of RELA and NFkB1 are induced, while, RELB, NFkB2, and the pluripotency factors OCT4, NANOG, SOX2, and KLF4 are downregulated. Moreover, chromatin immunoprecipitation (ChIP) revealed that RELA binds strongly to the regulatory regions of OCT4, SOX2 and KLF4 only when cells are differentiating, while, RELB is already bound to these regions in pluripotent undifferentiated cells. Here, we corroborate these findings using atRA-induced differentiation of H1 human embryonic stem cells (hESCs). By using quantitative automated fluorescence microscopy (High Content Analysis-HCA), we further show that siRNA-mediated knockdown of RELB in NTera-2 cells leads to a significant reduction in the percentage of OCT4-high pluripotent cells, from 80% in cells transfected with control siRNA, to 50% in siRNA-RELB transfected cells. Moreover, RELB was constitutively localized in the nucleus of H1 hESCs, while, RELA was found mainly in the cytoplasm. Lastly, transcript levels of OCT4, SOX2 and NANOG are downregulated in H1 hESCs upon RELB siRNA-silencing, while, RELA transcript levels are increased. Overall, our data strongly suggests that non-canonical NF-kB signaling mediated by RELB would act in the maintenance of pluripotency, while, canonical signaling trough RELA would be associated with differentiation by, respectively, promoting transcription or repression of key pluripotency factors.

Funding Source: São Paulo Research Foundation (FAPESP) and National Counsel of Technological and Scientific Development (CNPq).

F-2014

EXPANSION OF HUMAN PLURIPOTENT STEM CELLS AS AGGREGATES IN STIRRED TANK REACTORS; IMPROVED, SCALED, INTENSIFIED

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The commercialisation of allogeneic therapies derived from pluripotent stem cells (PSC) relies on the development and design of cost-efficient processing platforms suitable for the large-scale manufacture of medicinal products. The development and characterisation of appropriate upstream processes for PSC expansion has lacked utilisation of systematic methods to evaluate the relationship between process parameters, and product yield/quality. Here, we present The Cell and Gene Therapy Catapult (CGT) strategy for the development of a scalable and cost-efficient upstream process for the expansion of PSCs in high-density, aggregate based culture in stirred tank reactors (STR). We have applied a micro-bioreactor system to investigate the effects of impeller speed, seeding concentration, and feeding regime on the expansion of two PSC lines. Metabolism and growth rate data informed an expansion protocol targeting a 20-fold cell expansion over 5 days. Different impeller speeds were screened at a 10-fold higher scale to identify a suitable scaling factor between stirred tank reactors. The effects of type and concentration of dissociation agent on the time to achieve dissociation of cell aggregates were investigated for closed, in-vessel cell passaging, to assist further studies on continuous culture of multiple cycles

of expansion. Finally, integrated technologies are being explored for process intensification, concentration and washing. In addition to our laboratory based work, we are establishing a baseline risk/cost analysis model to assist process development for the large-scale expansion of PSC in STR. The model aims to provide an understanding of the process key cost drivers, and predict the impact of process changes on the risks and cost of GMP manufacture. Cost projections suggest the STR process could enable potential reduction in the fixed costs compared to traditional 2D adherent culture. The results of these collective studies will be used to establish a closed and scalable process for the intensified and cost-efficient expansion of PSCs as aggregates in stirred tank reactors. Proof-of-concept data for in-vessel closed processing suggests potential for the development of seamless expansion and differentiation processing platforms for the manufacture of PSC-derived allogeneic therapies.

Funding Source: The Cell and Gene Therapy Catapult works with Innovate UK

F-2016

AUTOPHAGY REGULATES AN EARLY CELLULAR STATE TRANSITION THAT RESETS PLURIPOTENCY IN HUMAN STEM CELLS

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Morphogenetic processes and cell-fate specification are intimately coupled in the early-embryo. Recent reports suggest human pluripotent stem cells recapitulate precise morphogenetic processes of early embryo development. Self-organizing human ES and iPS cells reproducibly generate spatiotemporal patterned domains that are biased towards mesendodermal or neurectodermal fates. The ability to interrogate key aspects of human embryonic development with genetic and chemical perturbations in these systems has generated much enthusiasm. To better define this process we have applied live-cell imaging and next generation RNA-sequencing to an in vitro paradigm of human morphogenesis. Three different cellular states that are spatially and temporally distinct were characterized in monolayer pluripotent cell culture. Cells in these different states show distinct transcriptional signatures, protein expression and differentiation potential. Because mTOR and RAPTOR deficient mouse embryos exhibit early post-implantation lethality we tested the hypothesis that the mTOR complex 1 inhibitor rapamycin would limit the progressive dynamics between distinct cell states. Rapamycin not only antagonized emergence of the later cell state primed towards anterior neural fates but also converted the entire epithelium to earlier less mature state. We also inhibited autophagic flux at various time points in pluripotency and found that autophagy was required at a specific point when pluripotency was reset following cell passage. These results define a new and central role for autophagy in the dynamic landscape of hPSC pluripotency. Autophagy has become an important cellular pathway for drug targets. Future studies will show how our pluripotency paradigm can be used to understand how drugs and genetic perturbations interact with the dynamics of tissue specification.

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F-2018

A SIMPLE CHEMICAL DEFINED AND FEEDER-FREE MEDIUM "STEMOTO MEDIUM" FOR HUMAN PLURIPOTENT STEM CELLS CULTURE

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The most common used human pluripotent stem cells (hPSCs) culture system is feeder cells dependent. To apply hPSCs for clinical use, a chemical defined and feeder-free hPSC culture system is trend to provide hPSCs a xeno-free and stable environment to growth. The most used commercial hPSC feeder-free culture medium mTeSR contain multiple components and high dosage FGF-basic (100 ng/mL), is a complex medium with high dosage growth factors. In our research, we found a small molecule Eco-002 and heparin could promote hPSCs proliferation at low dosage FGF-basic (10-20 ng/mL) and TGF-β1. Combining of these factors, we developed a light chemical defined medium named "Stemoto medium" for feeder-free hPSCs culture. This medium was a light and low protein medium that contained only 9 components. Stemoto medium could maintained not only Taiwan human embryonic cell (hESC) lines TW1 and hESC6, but also other standard hESC and induced pluripotent stem cell (iPSC) lines for long-term proliferation (including H9, H1, IMR90 and T21-094S1). After long-term culture in Stemoto medium, these cells presented normal hPSC morphologies, karyotype, proliferation rate and also expressed pluripotent specific markers. After differentiation test, we found these cells differentiated into three germ layers in vitro and in vivo teratoma formation test. With Sendai viruses reprogramming protocol, we

successfully reprogrammed human skin fibroblasts, bone marrow mesenchymal stem cells and peripheral blood mononuclear cells into iPSCs in Stemoto culture system. These reprogrammed iPSCs were with classic pluripotent stem cell morphologies, protein markers and in vitro differentiation abilities. To conclude our finding, Stemoto medium is a light, chemical defined medium for hPSC feeder-free culture and iPSCs establishment.

F-2020

ERV-BASED REPORTER PLATFORM FOR STUDYING EARLY HUMAN DEVELOPMENT IN VITRO

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Almost half of the human genome consists of transposable elements. Among these, endogenous retroviral elements (ERVs) are a class of retrotransposons representing remnants of infections that had occurred during early primate evolution. Most of the ERVs are thought to have lost their retroviral activity and therefore were considered dispensable for human development. However, emerging evidence links ERVs with shaping host gene regulatory networks while their mutations have been associated with immune diseases and cancer. Moreover, single cell transcriptomic analysis showed that specific ERV families are expressed during early human development in a stage-specific manner. These results suggest that ERVs might have a role during embryogenesis. Here, I attempt to establish a platform to study early human development by utilizing the stage-specific expression of ERVs. I intend to generate a panel of ERV-based fluorescent reporters that specifically label the distinct developmental stages in early human embryogenesis.

F-2022

CRYOPRESERVING AND THAWING CRYOPRESERVED HUMAN PLURIPOTENT STEM CELLS CULTURED IN STEMFIT®

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We have developed a defined, xeno-free culture medium, StemFit®, capable of robust feeder-free maintenance of undifferentiated human pluripotent stem cells (hPSCs). When used in combination with recombinant human laminin-511 E8 fragment, hPSCs could be easily and stably passaged as single cells, allowing for standardized expansion. Many hPSCs banks have been established around the world. We believe that it is necessary for these banks to create high quality stem cell stocks. In this study, we compare StemFit® medium with other commercial media in establishing cell banks. We investigate the relationship among the media used in cryopreserving and thawing cell stocks, cell stock manufacturing efficiency, and cell growth after thawing. Using two human iPSC lines: 201B7 and 1210B2 (each cell line established in Kyoto University), we investigated the effect of different media during hPSC cell stock creation. We cultured the cell lines in each medium, cryopreserved the cells, and then thawed and cultured them again in their respective medium. To validate the frozen cell stocks, we investigated the effect of the media on cell growth after thawing, and the stocks cultured in StemFit® were examined for growth over three passages. From our results, we report that culture media is one of the key factors that affect cell growth before and after cryopreservation. Furthermore, StemFit® was an appropriate medium for the establishment of robust and versatile hPSCs banks.

F-2024

QUANTITATIVE ASSESSMENT OF HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION POTENTIAL

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Human pluripotent stem cells (hPSC) play an important role in disease modeling, drug discovery and cell therapy applications. Today, generation of hiPSC has become a standard procedure in stem cell laboratories. Resulting cell lines need to be characterized after reprogramming and maintenance culture and must fulfill certain criteria, such as characteristic stem cell morphology, long-term self-renewal, karyotypic stability, expression of a specific marker profile, and differentiation capacity into all three germ layers. Differentiation capacity can be assessed in vivo using the Teratoma assay or in vitro using spontaneous or directed differentiation assays. Teratoma assays are hard to standardize and quantify, extremely costly and ethically controversial. Spontaneous differentiation assays in vitro are highly variable and also hard to read out. Evaluation of differentiation capacity is mostly done using qualitative immunocytochemistry since quantification requires sophisticated microscopy tools. Commonly used real-time PCR panels have the disadvantage of detecting only average gene expression values rather than revealing defined cell fates. To overcome these constraints, we aimed at developing a standardized, quantifiable differentiation assay based on lineage specific, complete media which support directed 2D differentiation in all three germ layers within 7 days. The assay format

allows quantitative flow cytometry analysis as well as immunocytochemistry assessment. As proof of principle four hiPSC lines were differentiated repeatedly and analyzed by flow cytometry and immunocytochemistry. The quantitative, flow-based analysis confirmed reproducible differentiation properties of all four hiPSC lines into Ectoderm, Mesoderm and Endoderm. Importantly, the assay revealed subtle differences in their intrinsic propensity to give rise to cells of the three germ layers, illustrating a convenient way to assess the differentiation potential of freshly reprogrammed hiPSC as well as established hPSC lines.

F-2026

THE IMPACT OF HIGH-DENSITY CELL CULTURE ON MAINTENANCE AND DIFFERENTIATION IN HUMAN PLURIPOTENT STEM CELLS

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Cell density has profound impact on cell growth, cell death and differentiation on human embryonic stem cells (hESCs). Much has been learnt in low-density culture, but it is still unclear how cells behave at high density. Chemically defined E8 medium was used to analyze cellular activities of hESCs at high density, and culture pH was identified as the main factor that affect cell culture consistency. The high cell density leads to cell culture acidosis, which in turn alters gene expression, cell cycle status and cell death. Adjustment of medium pH significantly affected cell survival, and plays important roles in ESC maintenance and differentiation. These results provide a new angle to improve stem cell maintenance and applications.

Funding Source: FDCT-131/2014/A3

F-2028

EXPLORING NANOG DEFICIENCY

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Nanog is a transcription factor important for regulating pluripotency, although established embryonic stem cells can self-renew in the absence of Nanog. Nanog is expressed in the early blastocyst and its role is crucial during embryogenesis due to the early lethality at E5.5, associated to its deficiency. Here, we have rescued this lethality and studied the consequences of severe Nanog deficiency in differentiated cells and in adult mice. Interestingly, Nanog-deficient fibroblasts cannot be reprogrammed into induced pluripotent stem cells, thereby, indicating a key role during the process of reprogramming. Adult Nanog-deficient mice do not have any major phenotype, however, they present impaired skin and hair regeneration. This suggests that endogenous Nanog plays an important role during reprogramming of differentiated cells, as well as, during skin and hair regeneration, and both processes could be mechanistically connected.

Funding Source: This project is funded by the European Research Council (ERC Advanced Grant) and the Spanish Ministry of Economy.

PLURIPOTENT STEM CELL DIFFERENTIATION

F-2032

SYNERGISTIC GROWTH FACTOR SIGNALING PROMOTES HUMAN AND MOUSE LUNG EPITHELIAL PROGENITOR CELL MAINTENANCE AND GENERATES LUNG PROGENITORS FROM HUMAN PLURIPOTENT STEM CELLS

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The developing lung forms a tree-like network of epithelial tubes that are comprised of progenitor cells, which later give rise to the proximal conducting airways and the distal alveoli, where gas exchange takes place. Many signaling pathways are known to be involved in lung development, however, it is currently unclear how these signaling pathways interact to regulate lung epithelial progenitor states and lineage fate choices. Furthermore, it is not known whether the mechanisms regulating murine lung development are mirrored in the developing human lung. To address these questions, we have used embryonic mouse and human tissue to identify growth factors that promote maintenance of distal lung epithelial progenitor cells in long-term, 3-dimensional culture. By performing a low throughput screen, we have identified growth factors that promote proliferation/expansion, and we have identified synergy between multiple pathways that are required to maintain expression of the distal progenitor cell identity. Our work has identified significant functional and molecular differences when comparing isolated human and mouse lung buds grown in vitro. Interestingly, we found that the same conditions that supported in vitro growth of primary mouse and human progenitor cells were able induce a distal epithelial lung progenitor-like state in human pluripotent stem cells (hPSCs). hPSC-derived

distal lung progenitor-like cells were maintained for over 100 days in vitro. hPSCs grown in these conditions gave rise to proximal-distal patterned human lung organoids (HLOs) with progenitors located in peripheral budding regions and interior regions containing mature secretory cells, reminiscent of the native proximal airway. Taken together, our studies have identified mechanisms controlling the distal epithelial progenitor cell state in developing mouse and human lungs, and we have demonstrated that the signaling mechanisms regulating in vivo biology can used to predict conditions that will control cellular decisions in differentiating hPSC culutres. hPSC-derived lung organoids with proximal and distal epithelial progenitors may prove to be an enabling tool to study lung epithelial branching and progenitor differentiation in a human specific model to enhance our understanding of human development and disease.

F-2034

A DEFINED AND SERUM FREE MEDIUM REVEALS THE ROLE OF RHO/ROCK SIGNALING IN TROPHOBLAST DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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The trophectoderm layer of the blastocyst stage embryo is the precursor of all trophoblast (TB) cell types in the placenta. Early human TB development is poorly understood due to legal and ethical constraints on research with human embryos and limited availability of placental samples from very early gestation. In this context, the use of TB derived from human embryonic stem cells (hESCs) as a model system has gained significant interest. Yet, whether hESC-derived cells are bona fide TB has been subject to rigorous debate over the years. We have previously shown that bona fide TB, as assessed using a stringent set of criteria, can indeed be obtained from hESCs (Sarkar et al., J. Biol Chem 2015 290(14):8834-48). Further, we showed that Activin/Nodal signaling acts in a switch-like manner to control the terminal differentiation of hESC-derived TB; syncytiotrophoblast (STB) is obtained in the presence of Activin/Nodal signaling, while Activin/Nodal inhibition results in selective differentiation to invasive trophoblasts (iCTBs) in 2D cultures. Despite these and other advances, a lack of mechanistic understanding of the process of TB fate commitment of hESCs has limited the widespread acceptance of the hESC-model of TB development. Importantly, non-availability of a completely defined, serum-free culture system for TB differentiation of hESCs, including terminal differentiation, is a major impediment to studies on the mechanistic basis of TB differentiation of hESCs. Towards this end, here we report the development of a completely defined and serum free medium for TB differentiation of hESCs. We show that terminally differentiated iCTBs and STB can be obtained, using similar assessment criteria for TB as previously discussed (Sarkar et al., J. Biol Chem 2015 290(14):8834-48). Further, using this system, we show that Rho/ROCK signaling is necessary for TB fate commitment of hESCs in this medium. Our studies will enable reliable generation of bona fide TB from hESCs, and mechanistic studies on downstream differentiation to terminally differentiated TB subtypes.

F-2036

SINGLE CELL RNA-SEQ AND A GENETICALLY ENCODED VOLTAGE INDICATOR REVEAL MOLECULAR AND FUNCTIONAL HETEROGENEITY OF HIPSC-CMS

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide a versatile tool for modeling cardiac development and disease. However, a number of challenges face this platform, including the ability to create and efficiently characterize pure and appropriately mature populations of cardiomyocyte subtypes. Genetically encoded voltage indicators (GEVIs) such as Arclight have shown potential as a means of assessing cardiomyocyte subtypes based on action potential morphology. Additionally, GEVIs are adaptable for screening in a higher throughput and less invasive manner than patch clamping. We sought to evaluate whether variation in action potential morphology reported by Arclight is concordant with gene expression and ion channel activity associated with maturation and subtype specification in hiPSC-CMs. To this end, we first characterized temporal changes in Arclight-reported electrophysiology and qRT-PCR gene expression over the course of hiPSC-CM differentiation. These assessments suggested the emergence of more defined subpopulations of hiPSC-CMs after approximately one month in culture. Furthermore, we demonstrated that the Kv1.5 inhibitor DPO-1 could be used to define atrial- versus ventricular-like action potential morphologies using both patch clamp and Arclight. In order to better profile the subgroups present at the gene expression level, we also performed single cell RNA-seq at D12 and D40 of differentiation, following characterization of those populations with Arclight. We were able to detect gene expression differences between the time points which supported the D40 cells having a more mature phenotype. Surprisingly, we observed a notable co-expression of standard markers for atrial, ventricular,

nodal, and conduction cell types at both time points, thus cells could not be distinctly delineated into a single subpopulation. Altogether, the Arclight and sequencing results highlighted the cell-to-cell heterogeneity present within the hiPSC-CM system. These findings suggest that well-defined cellular subtypes do not necessarily exist in a population of hiPSC-CMs, and that using electrophysiology alone or in conjunction with limited gene expression profiles can be insufficient for identifying distinct hiPSC-CM subtypes.

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F-2038

BREAKING THE EPIGENETIC BARRIERS THAT RESTRICT HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION

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Directed differentiation of human pluripotent stem cells (hPSCs) is a promising approach for cell replacement therapy and disease modeling. However, effectively differentiating hPSCs into different lineages, particularly into terminally differentiated cell types, has proven to be challenging. A number of studies have found that only certain stem lines have the capacity to differentiate into particular lineages due to epigenetic barriers that restrict multilineage differentiation potential. Here, we show that these epigenetic barriers are malleable and can be removed to promote terminal differentiation into many lineages. Using RNA-sequencing (RNA-seq), ChIP-sequencing, ATAC-sequencing, and DNA methylation analyses in hPSC lines in combination with directed differentiation, we demonstrate that propensity for differentiation is highly dependent on the starting state of hPSCs prior to directed differentiation and not necessarily due to the cell line itself. We show that there are variations in differentiation potential and epigenetic properties even within a cell line simply by altering the starting state. These variations are primarily due to differences in cell cycle properties. Using a Fluorescence Ubiquitin Cell Cycle Indicator (FUCCI) hPSC line, we dissect out the genetic and epigenetic properties and chromatin states characteristic of the early G1, late G1, and SG2M phases in hPSCs that have a high and low capacity for differentiation by performing RNA-seq and ATAC-seq in cell cycle sorted cells. In hPSCs that have a high capacity for differentiation, many cell cycle checkpoint controls have increased expression and chromatin accessibility (including Septins, Rb1, and ANAPC1), genes in the mini chromosome maintenance complex (MCM) family and POLA2 are enhanced and activated in a cell cycle phase specific manner, and many early developmental genes (e.g. Lefty1, Nodal) have an open chromatin state prior to gene activation. Using these mechanistic insights, we have developed molecular tools that enhance the differentiation of hPSCs into multiple lineages (including oligodendrocytes, pancreatic cells, and cardiac cells) with a high capacity for cell fate commitment and maturation into terminally differentiated cells for applications in regenerative medicine.

F-2040

HUMAN DEFINITIVE HEMATOPOIETIC SPECIFICATION IS REGULATED BY CDX4 EXPRESSION WITHIN MESODERM

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The generation of hematopoietic stem cells (HSCs) from human pluripotent stem cells (hPSCs) is a major goal for regenerative medicine. Achieving this goal is complicated by our incomplete understanding of human hematopoietic ontogeny, which consists of at least two distinct hematopoietic programs during development, each with different signal and genetic requirements. We have recently developed a novel method to obtain and identify, via stage-specific WNT signal manipulation, mesoderm harboring exclusively primitive or exclusively definitive hematopoietic potential, by the differential expression of CD235a within KDR+ mesoderm. Using this system, we performed whole-transcriptome gene expression analyses on WNT-dependent KDR+CD235a- definitive hematopoietic mesoderm and WNT-independent KDR+CD235a+ primitive hematopoietic mesoderm, and found strong CDX gene enrichment exclusively within KDR+CD235a- definitive hematopoietic mesoderm. Temporal expression analyses revealed that CDX4 was expressed exclusively within definitive hematopoietic KDR+CD235a- mesoderm in a WNT-dependent manner, suggesting CDX4 regulates definitive hematopoietic specification. Using hPSCs with inducible CDX4 expression via the AAVS1 locus, we found that exogenous CDX4 expression exclusively during mesoderm specification recapitulated the same effect as WNT stimulation during hPSC differentiation, with a >90% repression in primitive hematopoietic potential, and a 10-fold increase in definitive hematopoietic potential. In contrast, CRISPR/Cas9 generated CDX4

knockout hPSCs had intact primitive hematopoietic potential, but exhibited a 10-fold decrease in definitive erythro-myelo-lymphoid hematopoietic potential, with a 5-fold decrease in hemogenic endothelium. Taken together, these findings indicate that CDX4 is a critical transcription factor in the regulation of human definitive hematopoietic specification, and provides a mechanistic basis for WNT-mediated definitive hematopoietic specification from hPSCs. By understanding the genetic regulation of early definitive hematopoietic specification from hPSCs, we can now identify the additional signal pathways required for efficient HSC generation from hPSCs.

F-2042

MODELING OF BONE REMODELING BY THREE-DIMENSIONAL CO-CULTURE OF MOUSE EMBRYONIC STEM CELL-DERIVED OSTEOBLASTS AND OSTEOCLAST PRECURSORS

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Bone is maintained by several specialized cells through a process called bone remodeling. In this process, osteoblasts and hematopoietic lineage-derived osteoclasts communicate with each other through bi-directional signaling that regulates their functions. Osteoclastogenesis and the activation of osteoclasts largely depend on their interactions with osteoblastic cells through direct contact and paracrine factors; functional osteoblasts have the ability not only to secrete bone matrix but also to support osteoclastogenesis in vivo. We have developed culture strategies for differentiating pluripotent stem cells (PSCs) into osteoblastic cells. A bone synthesis-related function of PSC-derived osteoblasts has been extensively demonstrated both in vitro and in vivo, whereas multicellular interactions remain unexplored. Here we developed a three-dimensional (3D) co-culture system allowing osteoblast differentiation and subsequent interaction with osteoclast precursors. First, 2.3-kb Col1a1-GFP mouse embryonic stem cells (ESCs) were differentiated into osteoblastic cells within atelocollagen porous scaffolds by using small molecule inducers under defined conditions. Gene expression, protein expression and histological analyses revealed the formation of calcified structures containing osteoblast/osteocyte populations. Importantly, they highly expressed Rankl, a key stimulator of osteoclastogenesis. Osteoclast precursors from bone marrows of Rank-Cre;Rosa26-tdTomato mice were then seeded in the 3D culture system. After 1 week of the co-culture, TRAP-positive multinucleated cells were detected. Observation by two-photon microscopy revealed direct contact between green fluorescent protein-positive osteoblastic cells and tdTomato-positive osteoclastic cells as well as mature bone-resorbing osteoclasts. The present strategy may thus provide a system that reconstitutes the bone microenvironment in a 3D manner. Our data further suggest that a PSC-derived osteogenic population has not just osteogenic capacity to form bone, but also supports osteoclastogenesis. This strategy will allow us to reproduce and visualize the bone remodeling process in vitro in real-time and thus potentially elucidate unknown intercellular events during the bone remodeling process.

F-2044

A NOVEL 3D SPHEROID CULTURE SYSTEM FOR GENERATING FUNCTIONAL PANCREATIC &BETA; CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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A large-scale culture system for generating pancreatic β cells derive from human induced pluripotent stem cell (hiPSC) using spinner flasks has been reported. To improve the maturity of the hiPSC-derived pancreatic cells, we aim to optimize the culture procedure. We tested a variety of culture conditions, including medium composition that our group developed and combining a three-dimensional (3D) culture procedure reported by other group. We attempted to differentiate pancreatic β cells from hiPSC using a rotating culture system in 6-well plates. Here, we report the establishment of a novel five-step rotating culture system, generation of functional pancreatic β cells from hiPSCs. In addition, in our 3D rotating culture, we successfully produced insulin-positive cells up to 60%. We also found that 3D culture derived cells showed better response to glucose, compared to conventional two-dimensional cultures. Therefore, our results demonstrate that it is feasible to generate functional pancreatic β cells using a five-step culture system, and that this improved culture system

can be used for drug discovery of diabetes mellitus and as a tool for studying developmental biology of pancreas in humans.

F-2046

BETA-CATENIN ASSOCIATED PROTEIN COMPLEX MAINTAINS GROUND STATE MOUSE EMBRYONIC STEM CELL BY REGULATING GERMLINE DEVELOPMENT

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Mouse embryonic stem (ES) cells cultured in defined medium with MEK and GSK3 inhibitors(2i) resemble the pre-implantation epiblast in the ground state, with full development capacity including the somatic lineages and the germline. Although beta-catenin is known to be crucial for naive pluripotency of ES cells, the mechanism is not fully understood. Here I show that beta-catenin interacts with a repressive protein complex to maintain the ground state of ES cells by fine-tuning the germline development potential of ES cells. Absence of beta-catenin impairs ES cell self-renewal without affecting the core self-renewal circuitry of Oct4, Sox2 and Nanog as well as other pluripotency factors. However, beta-catenin-deficient cells show a primed state transcriptional signature with perturbed gene expression of germline and neuronal lineage. Knockdown of Tcf7l1, the repressor in canonical Wnt signaling pathway, does not completely rescue the beta-catenin-deficient phenotype of ES cells. Mechanistically, beta-catenin forms a novel biochemical complex with E2F6, HP1 gamma and HMGA2 to restrain ES cells from differentiation by co-occupying the promoter of germline and neuronal lineage regulators independent of TCF7L1. Moreover, beta-catenin functions differentially in early and late germ cell development, and keeps balance with E2F6 to prevent premature meiosis initiation in ES cells. Overall, my study shows that b-catenin forms a repressive protein complex with E2F6, HP1 gamma and HMGA2 to maintain ground state by orchestrating the development plasticity of ES cells.

F-2048

DISPARATE RESPONSE OF HUMAN EMBRYONIC STEM CELLS TO CHANGING ACTIVIN AND BMP4 CONCENTRATIONS REVEALS ROLE OF LIGAND DYNAMICS IN CELL FATE DETERMINATION

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How ligand dynamics affect cell fate is largely unknown, and this aspect of differentiation is often neglected in protocols for directed stem cell differentiation or models for embryogenesis on which these protocols are based. We address the role of ligand dynamics for the BMP and Activin/Nodal pathways, which are essential for directed differentiation to many fates, play crucial roles in gastrulation and mesendoderm differentiation in vivo, and share the signal transducer Smad4. Using live imaging of human embryonic stem cells with GFP integrated at the endogenous Smad4 locus combined with a fluidic system to create arbitrary ligand profiles in time, we demonstrate contrasting signaling response to BMP4 and Activin. We present our findings on the mechanism underlying this difference, and the consequences for cell fate.

F-2050

SINGLE-CELL ANALYSES IN STEM-CELL MODELS OF NEUROGENESIS REVEAL THAT CDK5RAP1 IS A CRUCIAL NEURAL SPECIFIC REGULATOR OF SOMATIC RETROTRANSPOSITION

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Retrotransposon-driven somatic mosaicism of Long INterspersed Element-1s (L1) is an endogenously encoded mechanism to generate genomic diversity in the brain. Somatic retrotransposition occurs more frequently in the brain than other tissue, and it has been suggested that properly tuned levels of retrotransposition are required for healthy brain development. Consistent with this hypothesis, somatic retrotransposition misregulation is associated with pathological conditions including rett syndrome, neurodegeneration and schizophrenia. Tissue specific regulatory mechanisms

are known to control retrotransposition in the germline; however, the cell-type specific dynamics and regulation of somatic retrotransposition in the healthy developing brain is largely unexplored. Herein, we determine the single-cell dynamics of somatic retrotransposition in developing neural progenitor cells. First to ask if specific populations of embryonic stem cell (ES) derived neural progenitor cells (NPC) have an increased propensity for retrotransposition, we determine how a previous retrotransposition event affects a NPC's propensity to complete a second somatic event. We discover that NPCs contain an autologous feedback mechanism such that once a neural progenitor cell undergoes retrotranpsosition it is less likely to undergo a second round of retrotransposition. Next, we used single cell transcriptome analysis combined with functional analysis of neural progenitor cells to discover which factors control this feedback effect. We identify Cdk5rap1 is a novel neural-specific regulator of somatic retrotransposition in the brain. Cdk5rap1 is known to inhibit cdk5, an essential kinase required for proper brain development and is altered in Alzheimer's disease. In addition, cdk5rap1 modifies RNA by adding a methylthio-group. We will present efforts to establish the mechanism by which cdk5rap1 regulates L1 retrotransposition in neural progenitor cells. These results demonstrate that somatic retrotransposition within the healthy developing brain is highly regulated and that neural progenitor cells invoke cell-type specific mechanisms to ensure proper levels of somatic retrotransopsition.

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F-2052

GENERATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED AIRWAY ORGANOIDS TO MODEL CYSTIC FIBROSIS IN VITRO

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Disorders affecting the lung epithelium, including cystic fibrosis, asthma, and chronic obstructive pulmonary disease, are responsible for a major burden of disease worldwide. However, these common afflictions remain poorly understood and treatment options for patients are limited. Rapid and reproducible derivation of functional airway organoids from human induced pluripotent stem cells (hiPSCs) represents a critical step towards disease modeling and drug screening for these and other airway disorders. However, an inadequate understanding of the signals regulating human lung patterning during development has made accomplishing this goal challenging. To test the role of developmental signaling pathways in hiPSC-derived lung patterning, we used a knock-in NKX2-1 reporter to track and purify early respiratory progenitors and evaluated the proximal airway vs. distal alveolar patterning response of these cells to activation or inhibition of predicted pathways. We found that Wnt signaling is a key regulator of proximodistal epithelial patterning in human NKX2-1+ lung progenitors. While Wnt activation is required for lung specification, withdrawal of this signal leads to rapid emergence of a proximal airway program and loss of distal identity. Building on this finding that stage-dependent modulation of Wnt activity promotes proximal lung fate, we have developed a novel protocol to rapidly and specifically differentiate epithelial-only airway organoids from hiPSCs. These organoids are derived from purified NKX2-1+ lung progenitors, contain key functional airway cell types including secretory, goblet, and basal cells, and can be further expanded and differentiated to multiciliated epithelia in air-liquid interface culture. To provide a proof of principle for the potential clinical utility of this platform, we generated airway organoids from cystic fibrosis patient-derived hiPSC lines pre- and post-correction of the dF508 mutation in the CFTR gene. These organoids respond in a CFTR-dependent manner to epithelial forskolin swelling assays, highlighting the potential utility of this approach for disease modeling and drug screening for a variety of genetic and acquired airway disorders.

F-2054

ROLE OF AUTOPHAGY IN HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION TO ENDODERM

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Autophagy (self-eating) is an important growth-regulatory mechanism involving recycling of damaged or dysfunctional cytoplasmic material. Animal models lacking essential autophagy genes are characterized by increased tumorigenesis and neurodegenerative phenotypes, indicating that autophagy has an important role both in tumor suppression and neuroprotection. A handful of studies have recently implicated a role for autophagy in differentiation. However, the molecular machinery, signaling events and cargo specificity involved are largely unknown. Here we demonstrate that autophagy is an important event in definitive

endoderm (DE) differentiation from human pluripotent stem cells. We show that several autophagic markers follow specific kinetics reflecting autophagic induction during differentiation into DE and find that inhibition of autophagy reduces the efficiency of differentiation. Moreover, we have preliminary evidence indicating that cargo involved in stem cell maintenance is targeted by autophagy. Our data is indicative of a role of autophagy in the specification of DE from human embryonic stem cells.

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F-2056

MICROGLIA-LIKE CELLS DIFFERENTIATED FROM INDUCED PLURIPOTENT STEM CELLS TREAT GLIOMA TUMORS IN MICE

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Glioblastoma Multiforme (GBM), a high-grade astrocytoma is the most aggressive primary brain tumor. In spite of multimodal treatment therapies of surgical resection, chemotherapy and radiotherapy, GBM patients have a very poor prognosis with median survival of ~16 months from the time of diagnosis. Recurrence is virtually inevitable and occurs at a median time of ~7 months. Among the more promising immunotherapy for recurrent GBM is the "vaccination" of patients with dendritic cells to elicit anti-tumor immune responses. The safety of this approach has been well demonstrated, but the clinical response rate of patients has ranged from 8%-30%. To improve upon these responses, we investigated the ability of microglia; the resident immune cells of the CNS, to better activate cytotoxic T lymphocytes within the tumor microenvironment. Using a murine syngeneic intracranial tumor model, we found that combined vaccination with dendritic cells and microglia is more effective than either alone. Combination treated mice had sustained regression of tumors over a four-week imaging period and the median survival of the animals was greater than one year. In contrast, the median survivals of the various control groups were ≤ 52 days. Translation of our findings to the clinical setting requires a source of patient-specific microglia. Here, we report the sequential differentiation of human induced pluripotent stem cells (hiPSC) into hematopoietic progenitor-like cells, and then into cells with a phenotype and gene expression profile resembling that of primary microglia. Similarly, hiPSC microglia-like cells (hiPS-MG) exhibit phagocytic activity; produce inflammatory cytokines and reactive oxygen species. We have also differentiated mouse iPSC into microglia (miPS-MG) with gene expression profile and phenotype similar to primary neonatal microglia. Mice treated with a combination therapy of tumor lysate-pulsed miPS-MG and dendritic cells show median survival greater than one year, whereas the survival for the control group was less than 50 days. For direct clinical translation of our findings, the ability to readily differentiate hiPSC into microglia in vitro will allow for the generation of patient-specific cells for use in a GBM immunotherapy protocol.

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F-2058

RAPID PRODUCTION OF NEURAL PROGENITOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS FOR THE STUDY OF ZIKA VIRUS NEUROPATHOGENESIS

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Zika virus (ZIKV) is an enveloped single-stranded RNA flavivirus that has been linked to fetal microcephaly and death. ZIKV preferentially targets neural progenitor cells (NPCs) and radial glia in two-dimensional and three-dimensional culture systems. The exact route of viral entry into NPCs, as well as the factors governing viral-mediated cell death, are unknown. High-throughput genetic and drug screens are needed to thoroughly answer these questions, though the execution of such experiments is hampered by the high costs and time associated with generating stable cultures of NPCs from human pluripotent stem cell (PSC) starting materials. Here, we describe a novel method that produces ZIKV-infectable human NPCs from PSCs in 48 hours, which is significantly shorter than previously published protocols. Using SMAD signaling inhibition in combination with lentiviral-based doxycycline-inducible overexpression of the transcription factor neurogenin-2 (NGN2), we were able to produce cultures that contained 90-99% NPC-marker positive cells from both human embryonic stem cells and human induced pluripotent stem cells without the need for a FAC sorting purification step. These cells, termed "rapid neural progenitor cells" (rNPCs), are susceptible to ZIKV infection and viral-mediated cell death at levels that are in line with previous

experiments using established NPC differentiation protocols. Our rNPCs are proliferative and capable of differentiating into both neurons and astrocytes, while studies are ongoing to determine oligodendrocyte potential. Under low attachment conditions, rNPCs are capable of self-aggregating into neurospheres that express several NPC and neuronal markers within 7 days of formation, as revealed by single cell RNA sequencing analysis. Importantly, these neurospheres show cell death-mediated decreases in size in response to ZIKV infection in a dose-dependent manner. Together, our findings suggest that rNPCs can be used for both two-dimensional and three-dimensional model systems for investigations of ZIKV neuropathogenesis.

F-2060

GUIDING HEMATOPOIETIC CELL FATE SPECIFICATION OF HUMAN PLURIPOTENT STEM CELLS WITH THE CRISPR-DCAS9 SYSTEM

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Human pluripotent stem cells (hPSCs) can in theory give rise to all hematopoietic lineages, thereby offering opportunities for disease modeling, drug screening, and cell therapies. However, directed differentiation of hPSCs into hematopoietic lineages remains inefficient and yields functionally immature cells. Transcriptomic analysis of hPSC-derived hematopoietic progenitor cells (HPCs) revealed that hundreds of genes are differentially regulated compared to native hematopoietic stem cells (HSC). We hypothesize that a subset of these genes are important for specifying the HSC fate, and that by reestablishing the endogenous expression pattern of these genes, it is possible to convert hPSC-derived HPCs into bona fide HSCs. The traditional overexpression system is not ideal for this purpose. This is because, 1) it overrides regulatory mechanisms of the endogenous locus (i.e. isoforms and post transcriptional regulation), and 2) it lacks the ability to accurately control the timing and the level of expression. Here we established a dCAS9-based inducible system for temporally controlled multiplexed transcriptional modulation during hPSC differentiation. Using this system, we activated genes involved in the specification of the hemogenic endothelium or in signaling pathways, such as Wnt, Activin and BMP, which are important for hematopoietic differentiation, during specific windows of differentiation. We show that the dCAS9 system is able to enhance hematopoietic cell fate specification of hPSCs. This new system could be exploited to reconstitute the authentic HSC gene expression program in hPSC-HPCs.

Funding Source: King Abdullah University of Science and Technology

F-2062

INTRACELLULAR BIOENERGETIC REDISTRIBUTION VIA HUMAN AK2 CONTROLS THE FATE OF HEMATOPOIETIC PROGENITORS

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Adenosine triphosphate (ATP) is an essential energy currency in cells. Since the main sites of ATP production dynamically transition during cell differentiation, intracellular energy redistribution is important for maintaining the appropriate energy level of each organelle. However, the contribution of key molecules that are involved in metabolic communication among subcellular components to the fate of progenitor cells remains unclear. AK2 is an adenylate phosphotransferase that is localized at the intermembrane spaces of the mitochondria. Although AK2 mutations can cause a form of severe combined immunodeficiency with neutropenia (reticular dysgenesis [RD]), the underlying mechanisms linking the AK2-mediated intracellular energy distribution to the fate decision of hematopoietic progenitors have not been fully elucidated. To address this issue, we used RD patient derived induced pluripotent stem cells (iPSCs) as a model of AK2 deificient human cells and compared with AK2 supplemented isogenic clones. The hematopoietic differentiation from RD-iPSCs was profoundly impaired in maturation of neutrophil and expression of T-lymphocyte lineage markers. Focused on early hematopoietic progenitor cells (HPCs), CD34+, CD43- RD-HPCs was severely defective in hematopoietic clonogenicity and maturation of mitochondria. Moreover, we assessed bioenergetic distribution in RD-HPCs. FRET-based single organelle live-imaging revealed the RD-HPCs had decreased ATP distribution in the nucleus while maintaining a higher level of mitochondrial ATP, despite the normal level of whole-cell ATP. The transcriptional profiles of RD-HPCs were globally altered with slower mRNA decay (42% decreased) and synthesis rate (23% decreased). The alternative enhancement of phosphotransfer by exogenous AK1, an isoenzyme of AK2, ameliorated the

hematopoietic defects. Thus, AK2 has a stage-specific role in maintaining the ATP supply to the nucleus during hematopoietic differentiation, which affects the transcriptional profiles necessary for controlling the fate of multipotential hematopoietic progenitors.

F-2064

AN ISY1-MEDIATED MIRNA BIOGENESIS PATHWAY CONTROLS 'POISED' PLURIPOTENCY

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microRNAs (miRNAs) are required for embryonic stem cell (ESC) self-renewal and pluripotency. ISY1 is implicated in the posttranscriptional control of miRNA expression yet its widespread role in miRNA regulation and relevance to ESC biology remain unknown. Here we find that ISY1 controls the expression of a large subset of miRNAs, and that biogenesis of a progenitor-miRNA (pro-miRNA) intermediate is a key step controlling expression of certain miRNAs. A transient peak in ISY1 levels during an early ESC transition defines a novel phase of pluripotency characterized by distinct miRNA and mRNA expression signatures. Loss- and gain-of-function experiments in ESCs reveal that ISY1 is necessary and sufficient to induce and maintain this 'poised' pluripotent state. Specifically, while transient ISY1 expression promotes exit from the naïve state and reprograms ESCs to poised pluripotency, persistently elevated ISY1 expression blocks ESC differentiation. Thus, dynamic ISY1 expression regulates miRNA levels and controls ESC pluripotency.

F-2066

HUMAN ATRIAL AND VENTRICULAR CARDIOMYOCYTES DEVELOP FROM DISTINCT MESODERM POPULATIONS

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Although it is well established that retinoic acid (RA) signaling plays a key role in specification of atrial cardiomyocytes (CMs), the target progenitor population that gives rise to this subpopulation of myocytes has not been characterized. To address this question, we used the human pluripotent stem cell (hPSC) model to dissect early lineage decisions during the onset of cardiovascular differentiation. We found that RA signaling during a 48-hour window of mesoderm development specified an atrial fate that resulted in the generation of a population of MLC2V-NKX2-5+COUPTFII+ atrial CMs. Based on findings in model organisms, we hypothesized that the RA responsive mesoderm should display retinaldehyde dehydrogenase (RALDH2) activity required to convert retinol (ROH) to RA. Through appropriate staged manipulation of Activin A and BMP4 signaling at the early stages of differentiation, we were able to generate a RALDH2+ mesoderm population that gave rise to atrial CMs following treatment with ROH. Increased concentrations of Activin A specified a different cardiogenic mesoderm that expressed glycophorin A (CD235a) but not RALDH2. This CD235a+ mesoderm showed no response to ROH and generated MLC2V+NKX2-5+COUPTFII- ventricular CMs. Together, these findings demonstrate that human ventricular and atrial CMs derive from distinct mesoderm populations that are identified by expression of CD235a and RALDH2 respectively. Although the CD235a+ ventricular mesoderm did not respond to ROH, it did respond to RA and generate atrial CMs. Functional analysis revealed that this CD235a-derived population contained a lower proportion of cells that displayed atrial-like action potentials (62%) compared to the population generated from the RA-treated RALDH2+ mesoderm (86%). In the absence of RA signaling both the CD235a+ and RALDH2+ mesoderm generated MLC2V+ ventricular CMs. However the proportion of MLC2v+ CMs was significantly higher in the CD235a+-derived population (≥80%) than in the population generated from the RALDH2+ mesoderm (≤30%). Collectively, these observations demonstrate that the generation of optimal ventricular or atrial CM populations is dependent on induction of the appropriate mesoderm, which has important implications for future disease modeling studies and cell-based therapy approaches.

F-2068

VARIATION IN NEURAL TRAJECTORIES TRAVERSED BY HUMAN PLURIPOTENT STEM CELLS

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The early developmental variability between human pluripotent stem cell (hPSC) lines is a challenge to their use for disease modeling and cell therapy. To systematically define the variation, we established new tools to analyze differences between pluripotent lines as they first reveal their differentiation potential. We report here that hPSCs spontaneously self-organize to form an epithelium with distinct zones representing major embryonic axes and variation is sustained over long periods in culture as hPSCs traverse a cycle of transcriptional states in early choices between fore- and hind-brain fates throughout differentiation to post-mitotic neurons. We show that transcriptional variation defined at early times predicts distinct neural trajectories. Investigation of additional replicate lines from the same genome revealed distinct biases in fore- and hind-brain fates and in generating precursors for dorsal and ventral telencephalic excitatory and inhibitory neurons. Donor-specific transcriptional signatures shared between replicate hPSC lines and defined in the early PSC differentiation distinguished the adult cerebral cortex of the same individual. We propose that the genetic and epigenetic mechanisms regulating human neural diversity in health and disease may be defined by systematic analysis of hPSC variation in the major axes of brain.

F-2070

GENERATION OF CD45-POSITIVE HEMATOPOIETIC CELLS FROM HUMAN IPS CELLS IN VIVO IN OVINE FETAL LIVER

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Generating definitive and engraftable human hematopoietic stem cells (HSCs) from pluripotent stem cells (PSCs) has been a major challenge in hematology. We have tried to generate such cells from human iPSCs. To this end, we tried to differentiate human iPSCs to HSCs on murine stromal OP9 cells with multiple cytokine milieu. The hematopoietic transcriptional factors Brachyury, Etv2 and Runx1, and the surface markers PDGFRa and CD34 expressed during the differentiation as time went on, recapitulating the mouse in vivo hematopoietic development. However, the hematopoietic essential marker CD45 failed to express over further extended culture period. Therefore, we hypothesized some key environmental factors are missing in vitro, thus limiting hematopoietic differentiation from iPSCs. To test this hypothesis, we transplanted human iPSC-derived intermediates at day 6 of the in vitro differentiation into the liver of busulfan-conditioned fetal ovine (day 47 - 63, full term 147 days). On day 6, the expression of Etv2 was declining, Scl expresses at the highest level, and the expression of Runx1 was just rising. At 1 - 2 months post-transplantation, the transplanted cells expressed CD45 in the fetal liver. Notably, human CD45+ cells were also observed in the bone marrow of the fetuses, suggesting that the transplanted cells were homing from the fetal liver to the bone marrow, which occurs during normal development. At 3 months post-transplantation, when the transplanted ovine was born, human CFUs were detected in the bone marrow of the lambs at levels of 2.3% to 6.3% (n = 4) and they were still detectable at 2 years post-transplantation. Given that many researchers have long failed to generate human iPSC-derived engraftable HSCs in vitro, the data here imply that the in vivo microenvironment such as in the ovine fetal liver is required for the acquisition of long-term hematopoietic engraftment ability of human iPSC-derived HSCs. Molecules responsible for the hematopoietic engraftment remain to be elucidated.

F-2072

A G-RICH MOTIF IN THE LNCRNA BRAVEHEART INTERACTS WITH A ZINC FINGER TRANSCRIPTION FACTOR TO SPECIFY THE CARDIOVASCULAR LINEAGE

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Long non-coding RNAs (lncRNAs) are a growing class of regulators of gene expression with diverse roles in lineage commitment and differentiation, however, their mechanisms of action remain poorly understood. Our prior work identified the mouse lncRNA Braveheart (Bvht), which acts in trans to regulate cardiovascular lineage commitment. Given that lncRNAs are generally lowly conserved by sequence and that many of these transcripts are species specific, we hypothesized that RNA secondary structure is key for understanding their broader roles and mechanisms of action. We determined the secondary structure of Bvht using chemical probing methods and show that this transcript adopts a modular secondary structure that harbors a unique 5' asymmetric G-rich internal loop, termed AGIL. Remarkably, CRISPR-mediated deletion of the 11 nt AGIL motif (bvhtdAGIL) in ESCs prevents cardiovascular differentiation similar to loss of full length transcript. Further analysis revealed that the zinc finger transcription factor cellular nucleic acid binding protein (CNBP) specifically interacts with the Bvht AGIL motif. We further show that CNBP acts as a negative regulator of the cardiac developmental program and that genetic ablation of CNBP partially rescues the differentiation defect of bvhtdAGIL mutant cells, suggesting that Bvht functionally antagonizes CNBP to promote cardiovascular lineage commitment. Our study demonstrates that structural studies combined with genetic perturbation can identify functional lncRNA motifs, opening the door to understanding their broader functions across species and to leveraging noncoding RNA motifs as biological tools and therapeutics.

F-2074

HIGH EFFICIENCY DIFFERENTIATION OF FUNCTIONAL PRO-EPICARDIAL CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Epicardial cells originate from the proepicardial organ to form the epicardium, promoting myocardial wall compaction during early heart development. Its absence has proven to lethally affect developing embryos, manifested by an underdeveloped myocardial wall. To date, this critical developmental phenomenon has not been recapitulated in vitro to aid engineering of functional myocardial constructs. Here we differentiated human iPS cells to lateral plate mesoderm (LPM) using CHIR99021 for 48 hr, which downregulated pluripotent genes (Oct3/4, Sox 2, Nanog) and upregulated mesoderm genes (T, MXL, ISL1, Nkx2.5) at day 3. We also found >90% of LPM cells expressed PDGFRα, assayed by flow cytometry. To induce pro-epicardial cell (PEC) differentiation, LPM cells were then treated with BMP4, VEGF, and retinoic acid for 96 hr. After 7 days, 86.8 ± 7.1% of LPM-derived cells demonstrated nuclear-localized WT1 protein expression, along with significant upregulation of proepicardial genes (WT1, TBX18, TCF21, BNC) and downregulation of cardiac genes (TNNT1, Nkx2.5). By immunocytochemistry, generated PECs showed high expression of WT1, ZO1, and Tcf21 with < 1 % of CD31 and SMA, and no expression of cTnT. With defined medium, WT1+ PECs also formed spheres within 24-48 hr in spin culture, with minimal contamination of smooth muscle cells and endothelial cells. They retained their cuboidal phenotype and expression after being passaged and re-plated at high density. Conversely, low density re-plating drove spontaneous smooth muscle differentiation and downregulation of WT1 expression. Using cell migration assay, a higher number of WT1+SMA+ cells were observed within the void area after 2 weeks, while non-migrating PECs remained WT1+SMA-. Co-culture of PEC with iPS-derived cardiomyocytes (CMs) induced changes in CM morphology, forming multiple CM-islands organized within a monolayer of PECs within 7 days. Under longer culture duration, CMs appeared to elongate and migrate to form connecting fibers with adjacent CM-islands, creating a synchronously beating cardiac network. These events may further elucidate the role of PECs in CM compaction and alignment during development. In generating iPS-derived PECs, it is now possible to study their potential involvement in ex vivo morphogenesis of engineered myocardial constructs.

F-2076

HIGHLY PURE CARDIOMYOCYTES GENERATION AND THEIR USES IN CARDIAC PHYSIOLOGY RESEARCH

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Human Cardiomyocytes derived from iPSCs hold a great promise to disease modeling and cardiac toxicity. Here we present a highly efficient differentiation 2D protocol for viral and episomal iPSCs. After 30 days of differentiation, cells can be replated in 2D monolayers or 3D spheroids. More than 70% of our differentiations have 95% or more of purity seen through positive expression of cardiac Troponin T. We saw different cardiomyocytes subtypes depending how cells are plated, 1/3 of each subtype in 2D plating and 97% ventricular and 3% nodal in 3D plating. We show electrophysiological response to classical drugs as expected, we checked responses to beta-adrenergic, calcium, sodium, potassium receptors including the verification of the well know hERG/IK potassium receptor. We also show response in different calcium channels by calcium imaging. We next evaluated if theses cardiomyocytes respond to hypertrophic stimulation and we saw higher expression of genes related to cardiac hypertrophy. These cardiomyocytes were also used the evaluate doxorrubicin toxicity, we show they are affected by this drug. We checked if these cells could respond to isquemia/reperfusion process. We saw neutral and negative response evaluating by LDH activity and XTT toxicity respectively. Finally, we showed that these cardiomyocytes have glycolytic metabolism and we tested different maintenance media to change their metabolic profile to oxidative. Cardiomyocytes transfection rates for DNA and siRNA were 60% and 98% respectively. Taken together, these data suggest that our cardiomyocytes are a good and reliable tool for cardiac research.

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F-2078

IN VITRO CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MACROPHAGES

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Macrophages play an essential role in phagocytosis, immunity and have an anti-inflammatory role in a variety of tissue types throughout the body. Tissue-specific macrophage cells, such as microglia are found in the central nervous system. Human induced pluripotent stem cells (iPSCs) were differentiated to monocytes using a novel, serum-free and defined growth factor-based protocol. Monocytes were then plated (seeding density of 0.3 million cells/well) and differentiated to produce terminally differentiated Human iPSC-Derived Macrophages (Axol Bioscience) containing large vesicles. Both the iPSC-derived monocytes and macrophages were characterized using fluorescence-activated cell sorting (FACs) analysis, phagocytosis assay, reactive oxygen species (ROS) assay, TNF-alpha ELISA and a cytokine array. FACs analysis revealed that the cells were strongly positive for CD45, CD14, CD11b cell surface markers. The phagocytic activity of these markers was assessed by quantifying the percentage of positive cells having taken up one or more zymosan particles. The production of ROS was visualized using the nitroblue tetrazolium assay. The presence of 36 different cytokines was detected using a membrane antibody array. Following characterization and validation, it is conclusive that iPSC-derived macrophages can be used as a representative model to replace current primary sources and immortalized cell lines with the benefits of scalability and reproducibility.

PLURIPOTENT STEM CELL: DISEASE MODELING

F-2080

DIFFERENTIATION OF HUMAN HIPPOCAMPAL CA3 NEURONS FROM ESC/IPSC REVEALS HIPPOCAMPAL ACTIVITY DEFICITS IN SCHIZOPHRENIA

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Hippocampus is selectively vulnerable to many psychiatric and neurological disorders. For example, synaptic transmission deficits in hippocampal neurons have been implicated in schizophrenia and bipolar disorder. The availability of human ES and induced pluripotent stem cells offers an opportunity to generate lineage-specific neural cells to investigate mechanisms of human diseases in relevant brain regions such as the hippocampus. Here, we report differentiation of hippocampal pyramidal neurons and neuronal connectivity between them. We established a robust ES and iPS cell-based protocol to differentiate human CA3 pyramidal neurons from patterned NPCs. The differentiated neuronal population illustrates multiple CA3 cell classes including the lesser-known ones and represents subtype unique to humans. The differentiated CA3 neurons are electrophysiologically active and form a neuronal network with dentate granule neurons, in vitro, recapitulating the synaptic connectivity within the hippocampus. Importantly, multi-electrode array recording revealed a reduction in spontaneous neuronal activity in DG–CA3 co-culture derived from schizophrenia patients, underscoring the relevance of this model in studying diseases with hippocampal vulnerability.

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F-2082

PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES FOR DISEASE MODELING OF NOONAN SYNDROME WITH MULTIPLE LENTIGINES

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Noonan syndrome with multiple lentigines (NSML), formerly known as LEOPARD Syndrome, is a rare autosomal dominant inherited disorder whose major characteristic features include lentigines, craniofacial dysmorphism, myocardium or valve abnormalities, electrocardiographic conduction defects and deafness. Approximately 90% of NSML cases are caused by missense mutations in the PTPN11 gene which encodes the protein tyrosine phosphatase SHP2. In this study, we generated iPS cell lines from peripheral blood mononuclear cells (PBMCs) derived from three NSML syndrome patients with PTPN11 gene mutations and two normal (wild type) patients using Sendai-virus technique. The iPS cells were extensively characterized and showed standard iPS cell morphology, carried normal human karyotype and expressed major pluripotency protein markers. The lineage differentiation capability of all three iPS cell lines was further confirmed by the standard teratoma formation assay. We further differentiated the patient-derived iPS cells into cardiomyocytes for disease modeling.

Funding Source: National Institutes of Health Intramural research program

F-2084

HUMAN IPSC-DERIVED ENDOTHELIAL CELLS CAN DEVELOP INTO BRAIN-LIKE ENDOTHELIAL CELLS AFTER COCULTURE WITH PRIMARY HUMAN BRAIN CELLS

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The blood brain barrier (BBB) is a selective endothelial interface that controls trafficking between the bloodstream and the brain interstitial space. The healthy human BBB development, its maintenance, and its disease states are difficult and time-consuming to study in vivo, and model systems with high relevance are needed. In vitro models provide a promising platform for screening of brain-penetrating therapeutics and studies of mechanisms behind BBB disruption. However, present BBB models are commonly comprised of immortalized cells, preferably from human sources, and have been hampered by the limited cell availability and low model fidelity. Human induced pluripotent stem cells (hiPSCs) is a promising and infinite cell source that enables large-scale production of specialized human cells, for example brain endothelial cells, with high similarity to their in vivo counterparts. Thus, hiPSCs have a high potential to serve as an excellent infinite human cell source for in vitro BBB models. The first step towards a hiPSC-derived BBB model is to investigate whether the hiPSC-derived endothelial cells have the capacity to develop into brain endothelial cells with characteristic brain endothelial cell properties. Important brain endothelial properties include the ability to form a tight monolayer with restricted permeability that mimics the BBB. In this study the potential of hiPSC-derived endothelial cells were investigated by co-culturing these cells with primary human brain cells. Results show that hiPSC-derived endothelial cells that are selectively purified, and derived with defined factors, will develop features similar to brain-like endothelial cells when cocultured with primary human astrocytes and pericytes. Interestingly, our data indicate that hiPSC-derived endothelial cells to some extend show similar plasticity as their in vivo counterparts and provide a promising system for modeling the human BBB. Such a model will be useful for studies of the molecular mechanisms of BBB specification of the endothelium and will aid to elucidate important pathways involved in the formation and maintenance of the BBB. In addition, the model may be further developed to exclude primary cell types, and allow for high-throughput screening for evaluation of BBB penetration of novel drug substances.

F-2086

IN VITRO DISEASE MODELING OF THE MAPT R406W MUTATION USING PATIENT-DERIVED IPSCS

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Frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17) is a neurodegenerative disease genetically caused by mutations in the microtubule-associated protein tau (MAPT) gene, which encodes the tau protein, and is one of the most common types of early-onset dementia after Alzheimer's Disease (AD). Over 50 mutations on the MAPT gene has been reported to induce FTDP-17, and disease phenotypes vary between patients with different mutations. Among them, the R406W mutation is a missense mutation on exon 13 of the MAPT gene. Interestingly, patients with the R406W mutation have been reported to exhibit AD-like phenotypes: early memory impairment is a primary presenting feature, while the more typical FTDP symptoms, such as changes in social behavior and personality, and motor symptoms (Parkinsonism), are less predominant or not seen at all. To date, there is no treatment known to be effective for FTDP-17, including the R406W mutation. The disease pathology must be further elucidated in order to find a suitable candidate for drug treatment. The objective of this study is to recapitulate pathological features of the R406W mutation using an iPSC model and to evaluate the abnormalities the mutation induces. iPSC lines from patients with the MAPT R406W mutation were established. Clones from each patient was gene-edited using CRISPR-Cas 9 to establish isogenic control lines with the mutation corrected, as well as homozygous mutant lines, with the mutation introduced into the wild allele of the gene. Using a conventional monolayer method and a three-dimensional method, these iPSC lines differentiated into cortical neurons of the forebrain region. Using these iPSC-derived platforms,

tau abnormalities were found in samples derived from R406W patient lines.

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F-2088

EFFICIENT GENOMIC CORRECTION OF MUTANTS IN COMPOUND ALPHA- AND BETA-THALASSAEMIA MAJOR IPSCS USING CRISPR/CAS9 SYSTEM

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Thalassemia is the most common monogenetic disease in all over the world. β-thalassemia major patients suffered from severe anemia and need lifelong transfusion and iron removing therapy. The combinations of beta thalassemia and alpha thalassemia, which are quite often in south China, make the clinical symptoms more complex. Currently, allogeneic bone marrow hematopoietic stem cell transplantation is the best way to treat thalassemia, however, the short of suitable donor and high costs restrict its clinical application. The development of induced pluripotent stem cells (iPSCs) and gene editing technology afford a new resource for hematopoietic stem cell transplantation treatment for thalassemia. In this study, we generated 174 iPS cell lines from the amniotic fluid cells donated by prenatal diagnosis patients, including iPSCs with normal genotype and iPSCs with 19 different thalassemia genotypes. We also constructed the plasmids consisted of β-globin gene (HBB) or HBB-sgRNA, α-globin gene (HBA)2-sgRNA, HBB-Donor, HBA2-Donor, which were transfected into iPSCs by electroporation, resulting in the genetic correction of the mutations in HBB and HBA2 by CRISPR/Cas9 system after homologous recombination. One special case is the correction of an iPS cell line combined with HBB β41-42/β41-42 mutants and HBA Westmead heterozygous point mutant. A serial of stem cell identification experiments were performed, including stem cell markers expression, karyotyping, teratoblastoma formation and hematopoietic induced differentiation. It turned out that, after gene correction, the stem cell markers expression, genetic stability, teratoblastoma formation of iPSCs did not change, however, the hematopoietic lineage differentiation potential were rescued. The above results indicated that iPSCs and CRISPR/Cas9 system provide useful strategy for thalassemia, even the complex cases of combination of HBB and HBA mutants.

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F-2090

STRESS-RELATED MITOCHONDRIAL DYSFUNCTION IN PATIENT-IPSC DERIVED NEURAL MODELS OF AFFECTIVE DISORDER

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Mitochondrial dysfunction has been implicated in mood and anxiety disorders such as bipolar disorder, post-traumatic stress disorder, and major depressive disorder (MDD), as well as cross-disorder outcomes, such as suicide. However, largely because of the difficulty in translating a number of affective dimensions in animal models, including suicidality, the underlying molecular pathology remains unexplored. Here we capitalize on patient induced pluripotent stem cell methods to derive and study neural models from healthy control subjects and from individuals with mood disorders. We examined the impact of the stress-related glucocorticoid, glutamate, and ERK pathways on mitochondrial homeostasis and integrity in 3 lines derived from healthy control subjects. Preliminary analysis using Seahorse bioassays revealed dose-response adverse effects of dexamethasone on bioenergetic measures. We will further present results of experiments contrasting these phenotypes with those measured in age- and sex-matched MDD patients stratified for lifetime history of trauma, treatment resistance, or suicidality. By exposing mitochondria from vulnerable versus resilient subjects to pathophysiologically-relevant concentrations of stress hormones, we aim to generate an in vitro two-hit model (predisposition x environment) that allows identification and potentially rescue of mitochondrial phenotypes associated with psychiatric vulnerability.

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F-2094

GENOME EDITING OF IPSCS AS A UNIVERSAL TREATMENT FOR FAMILIAL TRANSTHYRETIN AMYLOIDOSIS

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Familial transthyretin amyloidosis (ATTR) is an autosomal dominant disorder that can result from over one hundred different point mutations in the transthyretin (TTR) gene. In ATTR patients, TTR protein is produced by the liver, misfolded, and deposited as amyloid fibrils in peripheral organs leading to cardiomyopathy and peripheral neuropathy. Survivability varies greatly depending on which mutation is inherited, with an average time of diagnosis to death of 5 to 10 years. While orthotopic liver transplant is an approved treatment for ATTR, donor organ shortages and high variability in patient outcome necessitate additional treatment options. As an alternative to present therapeutics that target a single point mutation, we set out to develop a novel, universal gene correction strategy to ameliorate all forms of ATTR. To accomplish this, our laboratory has generated a genetically diverse library of ATTR disease-specific induced pluripotent stem cell (iPSC) lines. This bank is coupled with a directed differentiation strategy that harnesses the flexibility of an iPSC-based system to allow for the generation of all tissue types involved in the disorder and the testing of novel therapeutic strategies in the genetic context of the patient. Utilizing gene editing technology, fluorescently labeled, wild-type TTR was inserted into exon 1 of endogenous, mutant TTR in iPSCs derived from a patient with the most proteotoxic TTR variant. Upon directed differentiation of the genetically "corrected" iPSCs into hepatocytes, mass spectrometric analysis of their supernatants revealed complete ablation of mutant TTR secretion, while wild-type TTR levels remained unchanged. To evaluate the therapeutic advantage of this approach, supernatants from corrected and non-corrected lines were incubated with iPSC-derived neurons. Annexin V staining revealed a marked decrease of damage in neurons incubated with supernatant from corrected hepatocytes when compared to uncorrected. This study provides proof-of-concept for an iPSC-based universal gene correction strategy for treating a complex, multisystemic disease. In addition to therapeutic potential, our TTR reporter line can serve as a valuable tool to study the protein's production in real-time, allowing the opportunity to interrogate its role in additional cell types.

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F-2096

N-BUTYLIDENEPHTHALIDE ATTENUATES ALZHEIMER'S DISEASE-LIKE CYTOPATHY IN DOWN SYNDROME INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONS

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Down syndrome (DS) patients with early-onset dementia share similar neurodegenerative features with Alzheimer's disease (AD). To recapitulate the AD cell model, DS induced pluripotent stem cells (DS-iPSCs), reprogrammed from mesenchymal stem cells in amniotic fluid, were directed toward a neuronal lineage. Neuroepithelial precursor cells with high purity and forebrain characteristics were robustly generated on day 10 (D10) of differentiation. Accumulated amyloid deposits, Tau protein hyperphosphorylation and Tau intracellular redistribution emerged rapidly in DS neurons within 45 days but not in normal embryonic stem cell-derived neurons. N-butylidenephthalide (Bdph), a major phthalide ingredient of Angelica sinensis, was emulsified by pluronic F127 to reduce its cellular toxicity and promote canonical Wnt signaling. Interestingly, we found that F127-Bdph showed significant therapeutic effects in reducing secreted Aβ40 deposits, the total Tau level and the hyperphosphorylated status of Tau in DS neurons. Taken together, DS-iPSC derived neural cells can serve as an ideal cellular model of DS and AD and have potential for high-throughput screening of candidate drugs. We also suggest that Bdph may benefit DS or AD treatment by scavenging Aβ aggregates and neurofibrillary tangles.

F-2098

A COMPREHENSIVE, ETHNICALLY DIVERSE LIBRARY OF SICKLE CELL DISEASESPECIFIC INDUCED PLURIPOTENT STEM CELLS

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Sickle cell anemia is a disease caused by a single point mutation in which adenine is replaced with thymine within the sixth codon. Corresponding to a single base change, glutamic acid is substituted with valine in the β-globin amino acid chain of hemoglobin resulting in sickle shape of red blood cell. This disease affects millions of people worldwide and is an emerging global health burden. As part of a large, NIH-funded NextGen Consortium, we generated a diverse, comprehensive and fully characterized library of induced pluripotent stem cells (iPSCs) from sickle cell patients with different ethnicities, β-globin gene (HBB) haplotypes and fetal hemoglobin (HbF) levels. The library of patient-derived iPSC lines provides a unique resource for studying novel, haplotype-specific polymorphisms that affect disease severity as well as developing patient-specific therapeutics for this phenotypically diverse disorder. As a complement to this library and as a proof of principle for future cell- and gene-based therapies, we also corrected the sickle hemoglobin (HbS) mutation from a patient-derived iPSC line using the CRISPR/Cas9 gene editing tool while maintaining its differentiation potential for erythroid lineage. All cell lines have been deposited in the WiCell Stem Cell Bank for distribution to the scientific community.

F-2100

ROLES OF BRAIN AND REPRODUCTIVE ORGAN-EXPRESSED PROTEIN IN DNA DAMAGE RECOGNITION AND REPAIR RESPONDS OF MOUSE EMBRYONIC STEM CELLS

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Pluripotent mouse embryonic stem cells (mESCs) can undergo self-renewal and long-term proliferation, together with the ability to differentiate into various cell types. External factors, such as gamma irradiation, can induce DNA double-strand breaks. Inability to properly repair the DNA lesion will lead to genomic instability and mutations. Consequently, the build-up of mutation in mESCs may lead to metastasis and developmental defect. Hence, it is essential that the genetic integrity is maintained to prevent accumulation of mutation in mESCs. Brain and Reproductive Organ-Expressed protein (BRE), also known as BRCC45, is a component of the BRCA1-A complex. It is involved in the recognition of DNA double strand break and assists in the initiation of DNA repair response. We have previously demonstrated that knocking-out BRE expression in mouse tail fibroblast impaired BRCA1-A complex recruitment which subsequently leads to premature cellular senescence upon gamma irradiation treatment. However, mESCs can undergo self-renewal and long term proliferation. Moreover, mESCs have a significantly shortened cell cycle compared with fibroblasts, with defected cell cycle checkpoint, than other normal differentiated cells. Distinctive types of DNA damage response and DNA repair mechanism might be found in mESCs. In order to study the role of BRE in mESCs, we have generated BRE knockout (BRE-/-) mESCs from blastocyst of BRE-/- mice. Our results show that γ-H2AX foci, a DNA damage marker, expression is higher in BRE-/- mESCs than in WT mESCs. We will in future investigate and elucidate how the DNA repair is affected in BRE-/- mESCs and would the buildup of mutations affect embryonic development.

F-2102

IN VITRO DISEASE MODELING OF ALVEOLAR PHENOTYPE CAUSED BY AP3B1 DEFICIENCY USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Hermansky-Pudlak syndrome (HPS) is a genetic disorder characterized by oculocutaneous albinism, prolonged bleeding and accumulation of ceroid-like material, and one of the life-limiting complications is pulmonary fibrosis. Among 10 subtypes known to date, HPS type2 (HPS2) is caused by the mutation of AP3B1 gene, encoding the beta-3A subunit of adaptor protein 3A (AP-3A) complex which is involved in intracellular membrane trafficking. In HPS2 patients with bi-allelic AP3B1 gene mutations, pulmonary fibrosis occurs even in their infancy. Although giant lamellar body degeneration in the alveolar epithelial type 2 (AT2) cells was documented in some HPS patients with pulmonary fibrosis, the detailed mechanisms underlying the pathogenesis of pulmonary fibrosis in HPS2 patients have not been studied well due to the difficulty in obtaining and culturing patient-derived primary AT2 cells. Recently, we have developed a method of inducing and culturing AT2-like cells in alveolar organoids derived from human induced pluripotent stem cells (iPSCs). To investigate pulmonary fibrosis in vitro, we first established patient-derived iPSCs from fibroblasts of an HPS2 patient and then differentiated them into the alveolar organoids. The appearance of lamellar bodies in alveolar organoids derived from HPS2 patient-derived iPSCs seemed to be abnormal compared with those from normal iPSCs. To confirm that the change was caused by AP3B1 deficiency, we also established the gene corrected iPSCs from patient-derived iPSCs by homologous recombination using CRISPR-Cas9 systems. The newly established gene-corrected iPSCs could be differentiated into alveolar organoids and the lamellar body abnormalities seemed to be recovered by gene correction. Ultimately, it is suggested that the lamellar body abnormality observed in AP3B1 deficient alveolar organoids might underlie the pathogenesis pulmonary fibrosis in HPS2 patients.

F-2104

VULNERABILITY OF PURKINJE CELLS GENERATED FROM SPINOCEREBELLAR ATAXIA TYPE 6 PATIENT-DERIVED IPSCS

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Spinocerebellar ataxia type 6 (SCA6) is a dominantly inherited neurodegenerative disease characterized by loss of Purkinje cells in the cerebellum. It is caused by CAG trinucleotide repeat expansion in CACNA1A encoding Cav2.1, a1A subunit of P/Q-type calcium channel. However, the pathogenic mechanism and effective therapeutic treatments are still unknown. Here, we have succeeded in generating differentiated Purkinje cells derived from disease-specific iPSCs utilizing self-organizing culture technologies. SCA6 Purkinje cells exhibit increased levels of full-length Cav2.1 protein but decreased levels of its C-terminal fragment and downregulation of the transcriptional targets TAF1 and BTG1. We further demonstrate that SCA6 Purkinje cells show vulnerability to thyroid hormone depletion, resulting in degeneration. This vulnerability can be suppressed by two compounds, thyroid releasing hormone and Riluzole. Thus, we have constructed an in vitro disease model recapitulating both ontogenesis and pathogenesis. This model may be useful for pathogenic investigation and drug screening.

F-2106

FUNCTIONAL HIPSC-HEPATOCYES GENERATED FOR DISEASE MODELING AND DRUG DEVELOPMENT USING A UNIVERSAL DIFFERENTIATION PROTOCOL

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Hepatocytes derived from human pluripotent stem cells (hPSC) have the potential to serve as a predictive human in vitro model systems for disease modeling and drug development studies, provided that they possess relevant hepatocyte functions. The liver performs over 500 functions and many in vitro models lack the ability to faithfully recapitulate these functions. In addition,

existing differentiation protocols have not been robust enough for use with multiple hPSC lines, further hampering the use of hPSC-derived hepatocytes. Therefore, we have developed a robust differentiation protocol which recapitulates in vivo liver development and allows derivation of hepatocytes from multiple hPSC lines. Of 25 different hPSC lines tested, all lines were efficiently differentiated into highly homogenous hepatocyte cultures that exhibit important adult hepatocyte features, such as substantial CYP activities, low expression of fetal genes, and high expression of adult genes. More importantly, hepatocytes derived from multiple hPSC lines show diverse CYP activity profiles, thus reflecting the inter-individual variation present in the population. To allow generation of panels of cryopreserved hepatocytes from multiple hPSC lines, we have also developed a cryopreservation method for hPSC-derived hepatocytes. Like their fresh counterparts, the cryopreserved hPSC-derived hepatocytes have substantial CYP activities in the same range as in human primary hepatocytes. Importantly, a novel maintenance medium significantly improves hepatocyte functions, such as albumin and urea secretion, gluconeogenesis, glycogen storage, LDL-uptake, and lipid storage. We show the utility of the cryopreserved hPSC-derived hepatocytes for chronic toxicity studies. These functions are maintained for up to two weeks, nearly an order of magnitude greater than primary hepatocytes which rapidly lose their functionality within days in conventional 2D cultures. Taken together, our robust differentiation protocol together with the improved maintenance medium allow the reliable generation of mature hepatocytes from multiple hPSC lines. This can provide an inexhaustible source of human hepatocytes for use in in vitro disease modeling, drug discovery, metabolism, and chronic toxicity studies.

F-2108

EXPANSION OF 3D HUMAN INDUCED PLURIPOTENT STEM CELL AGGREGATES IN BIOREACTORS WITH A CLINICAL GRADE CULTURE MEDIUM

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Human induced pluripotent stem cells (hiPSC) are attractive tools for drug screening and disease modeling as well as promising candidates for cell therapy applications. Here we present the development of a defined, feeder-free medium, without human- or animal-derived components. hiPSCs that are cultured in this medium for an extended period of time express expected stem cell markers, remain diploid, and can differentiate into cell types from the three germ layers. Using this complete, clinical-grade culture medium, eight different hiPSC lines that were expanded as a 2D monolayer (2D culture) maintain high expression of pluripotent stem cell markers and lack any expression of differentiation markers over the 12–20 passages tested. In addition, no karyotype abnormalities were reported for any of the tested cell lines. In order to generate clinically relevant quantities of hiPSCs—109 and beyond—it is essential to develop efficient, yet robust 3D suspension cultures maintaining the same stability as 2D monolayer cultures. Previous reports in the literature of suspension cultures have typically described a reduced growth rate compared to monolayer cultures with a final cell concentration of 1–2 million cells per milliliter. We demonstrate that our culture system supports large-scale, 3D, non-adherent expansion of hiPSCs in suspension culture in a perfusion bioreactor. Furthermore, by optimizing perfusion rates and dissolved oxygen levels, we were able to expand hiPSCs by 1100-fold within 3 passages over 11 days to a final concentration of 5 million cells per milliliter using our 3D suspension culture system. In summary, our clinical-grade culture system allows for efficient, robust, and scalable production of hiPSCs, thus facilitating the use of hiPSCs for research and large-scale 3D suspension clinical applications.

F-2110

PHENOTYPIC ANALYSIS OF HUMAN IPSC-DERIVED DOPAMINERGIC NEURONS HARBORING THE ALPHA-SYNUCLEIN A53T MUTATION

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Parkinson's disease (PD) is the second most common neurodegenerative brain disorder after Alzheimer's, affecting more than 10 million people worldwide with 60,000 Americans being newly diagnosed each year. The pathophysiological decline associated with PD is generally thought to be caused by an aberrant deterioration of dopaminergic neurons located in the substantia nigra. PD has clearly been linked to mutations occurring in several different genes including SNCA, which encodes the alpha-synuclein (a-syn) protein that is predominantly expressed at presynaptic terminals. The a-syn protein acts as the predominate aggregate within lewey bodies, a hallmark biomarker of PD. A mutation in a-syn at A53T renders the protein more susceptible to aggregation and accumulation, which has rendered this mutation to be highly penetrant and one of the most

widely studied PD mutations. Interestingly, the mutant threonine (T) is actually the murine wild-type residue at this position, bringing into question the relevance of this mutation in existing PD mouse models. The advent of induced pluripotent stem cell (iPSC) technology has allowed the opportunity to study disease-specific mutations (such as A53T) in physiologically-relevant human cell cultures. Furthermore, the rise of cutting-edge genome-engineering techniques enables the generation of isogenic controls for use in phenotypic screening and disease modeling. Here we harness the power of iPSC technique to investigate the A53T mutation and its aberrant activities within human, dopaminergic neuronal cell cultures. In this poster, we present data comparing human midbrain dopaminergic neurons derived from healthy (WT) and mutant (A53T) iPS cells. We evaluate gene expression, mitochondrial bioenergetics, calcium handling, and network-level electrophysiological behaviors of both healthy and mutant cell cultures. The observed differences in these assays suggest the a-syn A53T mutant displays early pathophysiological changes tilted towards a more connected, highly-active neuronal network. In correlation with the expected pathology, current studies are ongoing to determine if "aging" cultures display the disease-relevant synaptic deterioration, including dendritic atrophy, a-syn aggregation, and / or additional mitochondrial dysfunction.

F-2112

GENERATION OF FANCONI ANEMIA INDUCED PLURIPOTENT STEM CELLS FOR DISEASE MODELLING

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Fanconi anemia (FA) is caused by genetic defects in the Fanconi anemia pathway (FA-pathway), which is involved in DNA inter-strand cross-link repair. The disease is characterized by progressive bone marrow failure with a significant number of patients developing additional complications including leukemia, myelodysplastic syndrome and solid tumors. Somatic cells from FA patients are highly refractory to reprogramming, and gene correction to restore the functional FA pathway was found to be essential for the generation of FA-iPSC lines with normal karyotypes. For the derivation of an FA patient specific hiPSC line capable of mimicking the disease phenotypes in vitro, we used inducible complementation strategy. Dermal fibroblasts were obtained from an individual diagnosed to have FA based on the clinical features and a very high number of chromosome breakages in the peripheral blood lymphocytes after treatment with Mitomycin C. Complementation analysis was performed by transducing the fibroblasts with a DOX-inducible lentiviral vector encoding FANCA gene (pINDUCER20-FANCA), followed by western blot analysis for FANCD2 ubiquitination. Restoration of FANCD2 ubiquitination in these fibroblasts cultured in the presence of DOX, suggested that FANCA gene was defective in this patient. We reprogrammed the pINDUCER20-FANCA transduced fibroblasts using Sendai Viruses to express the reprogramming factors along with FANCA complementation in the presence of DOX. Six iPSC colonies were isolated based on the morphology and cultured in the presence of DOX. These iPSC lines expressed all the pluripotency markers and could form teratomas with three germ layers and had normal karyotypes. On DOX withdrawal, these cell lines showed the absence of FANCA expression and the features of FA cell features, i.e. the lack of FANCD2 ubiquitination, lack of γH2A.X-FANCD2 colocalization on DNA damage sites, and cell cycle arrest at G2/M phase leading to cell death and progressive exhaustion in culture. RNA sequencing carried out with one of these iPSC lines in the presence and absence of DOX identified several drug targetable pathways. Our results showed that these iPSC lines and the hematopoietic cells derived from them, in the presence and the absence of DOX, can be used for understanding FA disease mechanisms.

REPROGRAMMING

F-2116

GENERATING MULTIPOTENT STEM CELLS FROM PRIMARY HUMAN ADIPOCYTES

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Current trends in regenerative medicine focus on generating tissue specific stem cells to repair damaged tissues. However, given the complexity of most tissues, the ideal tissue regenerative stem cell would be one that was sufficiently plastic to contribute to the repair of multiple tissue types in a context dependent manner. We have developed a vector and transcription factor-free method using a demethylating agent (5'-azacitidine (AZA)) and a cytokine (platelet derived growth factor (Pdgf)-AB) to reprogram terminally differentiated somatic cells into multipotent stem (iMS) cells by synergistically activating the JAK/STAT and JNK/c-JUN pathways. Murine iMS cells contribute directly to in vivo tissue regeneration in a context dependent manner without scar formation or malignant transformation (Chandrakanthan et al. PNAS 2016). This Demethylation Cytokine induced (DCi) reprogramming method has now been modified to transform human primary adipocytes into iMS cells under xeno-free conditions. The resultant human iMS cells can be expanded in serum-free conditions and maintain a stable karyotype. Immunophenotyping showed positive expression of CD73, CD90 and CD105 with a lack in expression of CD14, CD20, CD34 and CD45 on these cells. Human iMS cells also display in vitro colony forming potential, serial re-plating ability and multi-lineage differentiation capacity. Tissue persistence and repair potential of transplanted human iMS cells were investigated using a postero-lateral inter-lumbar vertebral injury model in NOD/SCID mice. Periodic bioluminescence imaging revealed retention of human iMS cells at the transplant site for one year with no evidence of cell translocation or spontaneous teratoma formation. At 6 months post-surgery, human iMS cells were shown to contribute formation of new blood vessels, bone, cartilage and smooth muscle at the site of injury. Taken together, our data showed that DCi reprogramming is a safe method of generating tissue regenerative multipotent stem cells from terminally differentiated somatic cells and may provide an alternate route to therapeutic tissue regeneration. Investigation of plasticity of human iMS cells in a pluripotent context and their long-term stability when transplanted in tissue specific injury models are in progress.

F-2118

NOVEL MEASLES-BASED REPROGRAMMING SYSTEM: THE FUTURE FOR CLINICALLY APPLICABLE IPSC GENERATION

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Recent advancements in iPSCs technologies have offered potential application of iPSCs in many areas such as stem cell therapies. Several vehicles have been developed to deliver genes for reprogramming from integrating lentiviral, retroviral vectors, RNAs, proteins to plasmids. All these carriers were used to reprogram fibroblasts into iPSCs, but their efficiency to reprogram patient-derived cells were either low or the use of integrating vectors adds concern over increased tumorigenicity due to insertional mutagenesis. Although a non-integrating system based on Sendai virus has emerged as a promising reprogramming platform, its translation into patients is limited, as the safety of Sendai virus in humans remains to be determined. Thus, it is necessary to develop an efficient, alternative cellular reprogramming system for clinically applicable, transgene-free iPSC derivation. In this study, we present a novel, one-cycle Measles virus (MV) reprogramming vector, based on a well-characterized, safe vaccine strain of MV. Specifically, we engineered the Moraten strain to express the four reprogramming factors (RF) from a single, replication-defective MV vector. This vector only propagates in a packaging cell line expressing MV hemagglutinin. A designed, polycistronic OSK sequence, encoding OCT4, SOX2 and KLF4, was cloned instead of the H gene, whereas cMYC ORF was inserted directly downstream of the OSK. The proper processing

and expression of the RF were verified by Western blot and immunofluorescence on transduced fibroblasts. Our single, polycistronic MV vector reprogrammed fibroblasts into iPSCs with expression of pluripotency-associated marker genes, such as SSEA-4, TRA-1-81, TRA-1-60, and Nanog, with comparable efficiency to our lentiviral RF vectors. Pluripotency of derived iPSC clones was confirmed by spontaneous differentiation and guided differentiation into three germ layers. Unlike Sendai vectors, MV vector genomes were rapidly eliminated from established iPSC within few passages. Given the excellent safety record of MV vaccines and the established safety of genetically modified MVs in cancer clinical trials, our single MV vector system provides an RNA-based, non-integrating gene-transfer platform for nuclear reprogramming that is amenable for immediate clinical translation.

F-2120

TRANSIENT CREB-MEDIATED TRANSCRIPTION IS KEY IN DIRECT NEURONAL REPROGRAMMING

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Combination of neuronal determinants and/or small-molecules such as forskolin can be used to convert different cell types into neurons. As Fk is known to activate cAMP-dependent pathways including CREB-activity, we aimed here to determine the role of CREB in reprogramming – including its temporal profile. First, we modulated the activation of CREB during astroglia-to-neuron conversion by exposing glial cells to Fk. We observed that treatment of astrocytes or MEFs with Fk induces a short-fast phosphorylation of CREB followed later by persistent dephosphorylation without affecting its protein levels. Second, to artificially control the temporal activity of CREB during reprogramming, we designed a vector based on the Tet-On system in which the cDNA encoding the dominant positive CREB-VP16 protein is only activated in presence of doxycycline. This approach, in combination with the constitutive expression of the dominant-negative ICER, allowed us to manipulate CREB activity in a temporal fashion similar to the effect of Fk and to demonstrate that the dynamic regulation with first increased and later decreased levels of P-CREB enhances efficiency of neuronal conversion of astrocytes mediated by Ascl1. Contrarily, persistent over-expression of either CREB-VP16 or ICER impedes reprogramming, indicating that transient CREB-mediated transcription is the correct road to activate a successful neurogenic program in astrocytes. In addition, we also found that both treatment with Fk or expression of CREB-VP16 increase levels of Bcl-2 protein in astrocytes, therefore, strongly suggesting that CREB and Bcl-2 may be critical mediators of the effect of Fk in neuronal reprogramming.

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F-2122

LARGE SCALE GMP MANUFACTURING OF INDIVIDUALIZED AUTOLOGOUS DIRECTLY REPROGRAMMED HUMAN NEURAL PRECURSOR CELLS FOR CLINICAL APPLICATIONS

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Direct cell reprogramming into neural precursor cells provides an attractive venue for clinical translation of autologous neural stem cell therapy for CNS indications such as stroke, spinal cord injury, traumatic brain injury and Parkinson's disease, without the use of fetuses, embryos or pluripotent cell intermediates. Transient expression of JA1, an optimized non-integrating plasmid containing three synthesized transcription factors (Musashi-1, Neurogenin-2 and Methyl-CpG Binding Domain Protein 2) has been previously shown to result in robust reprogramming of human somatic cells into neural stem cells, termed directly reprogrammed neural precursor cells (drNPCs), within 2 weeks at high efficiency. drNPC transplantation has shown significant efficacy in pre-clinical animal models and no tumour formation in pre-clinical toxicology / tumourigenicity studies, making drNPCs an attractive cell source for clinical translation. However, GMP manufacturing of an autologous directly reprogrammed cell therapy requires the development of new manufacturing methods to overcome the otherwise commercially unfeasible high costs of GMP manufacturing and operator batch-to-batch variability. A fully automated robotic GMP manufacturing system with on-board robotic QC capabilities and environmental systems to prevent any cross-contamination between continuous batches was built under contract with 14 different robotics and equipment manufacturers, with each system capable of manufacturing annually up to 10,000 individual patient batches of 120 million drNPCs per batch with virtually no operator batch-to-batch variability, all at a fraction of the cost of manual GMP manufacturing and QC analysis, and with minimal training of only a six operators (compared to 2,000+ highly trained operators that would be required for manual GMP production of 10,000 drNPC batches per year). Additionally the built-in FDA 21CFR.11 compliance and full automated traceability of all manufacturing and QC steps significantly reduces the

complexity and regulatory burden of reprogrammed cell manufacturing, opening this new stem cell field to more rapid clinical translation of cell therapies for clinical trials and commercialization. As a result, drNPC are the first reprogrammed cells to be manufactured under GMP at large scale.

F-2124

IDENTIFICATION OF CELLULAR PLASTICITY INHIBITORS BY SH RNA SCREENINGS IN MOUSE MAMMARY PROGENITORS

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The inter-conversion of cells between differentiated and stem cell behavior, cellular plasticity, is increasingly emerging as a physiological mechanism of tissue homeostasis, which allows tissue repair after injury. Cellular plasticity could also be involved in tumor initiation and maintenance, leading to the generation of tumor initiating cells, respectively, by de - differentiation of normal tissue, or from non - tumorigenic bulk tumor cells. Therefore, cellular plasticity must be tightly controlled to avoid unscheduled de-differentiation, but the genes involved are yet largely unknown. In order to identify physiological inhibitors of cellular plasticity, we screened lentiviral shRNA libraries in mouse mammary progenitors, to identify shRNAs endowing them with SC properties: self-renewal ability in vitro and regenerative potential in vivo. We screened pooled lentiviral shRNA libraries targeting 240 epigenetic regulators, each by 10 different shRNAs, by employing two sub-libraries of lower complexity, containing 1,200 shRNAs each. Mammary progenitors were purified by a well-established label - retaining protocol (PKH26) in mammosphere suspension culture, infected with the lentiviral sh libraries, and orthotopically transplanted into mouse mammary fat-pads, cleared of the endogenous mammary tissue. HTS of genomic DNA extracted from the regenerated mammary gland, led to the identification of candidate shRNAs for validations. We identified several candidates, and validated some of them in the same mammary regeneration assay.

F-2126

IMPROVEMENT OF RNA-BASED REPROGRAMMING USING STABILIZING 3'-UNTRANSLATED REGIONS OF SYNTHETIC RNAS

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Synthetic mRNA is the most favorable vector for reprogramming of somatic cells into induced pluripotent stem cells (iPS) since it does not integrate into the genome and therefore overcomes many limitations concerning clinical applications of iPS technology. We recently developed an mRNA reprogramming technology based on reprogramming mRNAs [OCT4, SOX2, KLF4, cMYC, NANOG and LIN28] as well as double stranded microRNAs from the 302/367 cluster. Although the mRNAs are non-modified and thus immunogenic, addition of immune evasion mRNAs [E3, K3 and B18R] from Vaccinia virus allows to generate robustly stable iPS lines from human neonatal and adult fibroblasts, human blood-derived endothelial progenitor cells (EPC) and human urine-derived epithelial cells (UDC) with only four (fibroblasts) to eight (EPC/UDC) daily transfections. In regard to clinical application of mRNA reprogramming and therefore the generation of GMP-grade iPS it is desirable and even inevitable to further simplify the technology. One approach is thereby to enhance the intracellular persistence of immanently unstable mRNA. Stability of mRNA largely depends on structural elements within the 3'-untranslated region (UTR). Using a Systematic Evolution of Ligands by EXponential Enrichment (SELEX) based approach we were able to identify a new 3'UTR consisting of two elements called F and I which stabilizes synthetic mRNAs and enhances their expression kinetic. When this 3' UTR was used to replace the original 3' UTR of reprogramming and immune evasion mRNAs - a tandem repeat of the human ß-globin 3' UTR - we were able to reduce the number of repetitive transfections for reprogramming of human fibroblasts down to only two transfections. Human embryonic stem cell (hES) like morphology and the expression of hES markers of resulting iPS colonies underline successful reprogramming of cells. Taken together our results show that further stabilization of synthetic mRNAs via optimized 3' UTRs leads to a more rapid and efficient reprogramming. With further improvements even a single mRNA transfection for successful reprogramming appears achievable. This could be especially beneficial in the context of clinical

translation and the attempt to generate GMP-grade iPS from refractory cell lines or cell types such as EPC or UDC that require multiple transfections.

F-2128

GENOME INTEGRATION-FREE METHODS FOR GENERATING HEPATOCYTE-LIKE CELLS

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Direct conversion techniques that differentiate from fibroblasts to target somatic cells are useful for skipping induced pluripotent stem cell stages. However, ectopic expression of transcription factor with retrovirus or lentivirus for conversion results in integration into host genome and limitation translational application. Here, we generate hepatocyte-like cells (iHeps) using mRNA transfection avoiding genome integration. Three hepatic transcription factors mRNA, HNF4a, Foxa3 and GATA4, are expressed into mouse embryonic fibroblasts (MEFs), and cultured with iHep media. Seven days after transfection, epithelial type cells are spread out from fibroblasts, and proliferated quickly and densely. These cells form epithelia colonies that are presented in hepatic characteristics, such as polygonal shape in a mono or bi-nucleus. And hepatic marker genes, which are albumin, AFP, ASGR1, etc., are expressed in iHeps. Functionally, glycogen synthesis and liver substance uptake are confirmed by periodic acid-Schiff (PAS) staining and indocyanine green (ICG) uptake.

F-2130

A NOVEL METHOD FOR PRODUCTION OF CLINICAL-GRADE INDUCED PLURIPOTENT STEM CELLS USING AUTOLOGOUS SERUM AND ANIMAL COMPONENT-FREE CULTURE CONDITIONS

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Translation of induced pluripotent stem cell (iPSC)-derived tissues into the clinic will require the streamlined production of clinical-grade iPSCs. We have developed an entirely xeno-free procedure to derive stable, monoclonal iPSC lines from donor blood using autologous patient material and defined reagents manufactured under cGMP. Peripheral blood mononuclear cells (PBMCs) are received in a whole blood donation from which a small amount of patient serum is also isolated. PBMCs are expanded prior to reprogramming in animal component-free erythroblast growth medium with autologous patient serum, mitigating the need for animal serum. After expansion, the erythroblasts are reprogrammed using the ThermoFisher Epi5 episomal reprogramming vectors. Resulting iPSC colonies are picked directly from a recombinant human laminin substrate and expanded in a serum-free, defined media, eliminating the need for feeder cells or animal-derived matrices. Monolayer growth on recombinant laminin enables very rapid and high-density expansion without the need to manually remove differentiated areas or split colonies. The relative ease of this method would make it quite feasible to perform the entire process inside a "cGMP in a box" type of setup. We developed highly sensitive qPCR and ddPCR assays to confirm the absence of episomal DNA sequences. The quality of resultant iPSC lines was further confirmed by genomic (G banding and CGH) analysis as well as by immunofluorescent staining for known pluripotency markers. Furthermore, the cells performed very well in directed differentiation experiments, including into hemato-endothelial and neuronal lineages. Through five independent derivation experiments we show this platform to be very robust and reliable. Our current work is focused on optimizing the reprogramming step of the protocol for highest efficiency. We will also present a comparison of several other episomal systems as well as non-integrating Sendai virus mediated reprogramming. Additionally, we will present preliminary results of ongoing attempts to also incorporate efficient CRISPR-mediated genomic repair into this clinical-grade hiPSC derivation and expansion platform.

F-2132

SMALL MOLECULE ACTIVATION OF PROGENITOR CELLS AS A MEANS OF IN SITU TISSUE REGENERATION

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The applicability of tissue regeneration is limited by the complexities of cell therapy, including cell delivery and engraftment. Small molecules can activate dormant progenitor cells at specific sites within the body. Called Progenitor Cell Activation (PCA), this approach may provide a novel means of addressing cellular deficiencies or malfunctions in many diseases including hearing loss, gastrointestinal disorders, and diabetes. Hearing loss

caused by damaged or killed sensory cells affects 36 million Americans. The cochlear cells expressing Lgr5 are known to give rise to sensory cells in development, but remain dormant thereafter, making any loss of sensory cells after birth permanent. A small molecule screening program utilizing cochlear Lgr5 progenitor cells has yielded the candidate FX322. The application of FX322 to cochlear stem cells from mice and humans resulted in cellular expansion and the creation of new hair cells with appropriate structural components and function. When applied to isolated cochleae ex vivo, FX322 generated additional hair cells and supporting cells. Further, FX322 improved hearing across all frequencies in mice that were deafened with loud noise. The same PCA screening platform was applied to Lgr5 expressing cells in other tissue types. Screening against Lgr5 stem cells from human and murine GI tracks yielded molecules that enabled the selective cellular growth and differentiation in high purity to cells types including goblet cells and Paneth cells which are important in the treatment of conditions such as IBD or microbiome disorders. Small molecule cocktails were found to produce high purity enteroendocrine cells from intestinal Lgr5 stem cells, including GLP1, GIP, and serotonin producing cells, which have important functions in digestive and metabolic disorders including diabetes. We believe PCA could be used to modulate cells in situ to address a number of diseases.

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TECHNOLOGIES FOR STEM CELL RESEARCH

F-2134

THE XENO-FREE STEM CELL INITIATIVE: CRITICAL RESOURCES FOR CLINICAL TRANSLATION AND AUTOLOGOUS MEDICINE

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Human induced pluripotent stem cell (iPSC) research has the great potential of yielding more human-relevant models of disease, replacing animal models in research and regenerating damaged tissues in patients. The transition to Xeno-Free Stem Cell (XFSC) science is also becoming urgent as regulatory bodies increase the focus on transparency and reproducibility. However, the generation and differentiation of iPSC in many studies are still contaminated with animal-derived products ("xeno-contaminants") which constitute a major bottleneck to clinical translation. Although many xeno-free approaches have been developed, these techniques are not readily accessible or widely adopted for use in research and clinical applications. Here we describe a XFSC initiative that aims to address these challenges and implement xeno-free methods more broadly. Specifically, we are developing the first online XFSC toolkit to provide detailed protocols and sources of XF reagents in standardized formats with advanced searching functions. The toolkit will allow users to report success of reproducibility, comment on troubleshooting issues and provide sample results. To date, we have catalogued more than 100 primary, peer-reviewed articles reporting XF iPSC techniques. We have also examined current guidelines and studies that adhere to Good Manufacturing Practices and compared the cost of currently available reagents and supplies necessary for XF study conditions with their animal-derived counterparts. Our preliminary findings show that, in many cases, the XF alternatives are already available and that they are essential when considered in the context of clinical trial requirements. The move to fully xeno-free research is also an essential step in addressing allogenic roadblocks to personalized medicine. By eliminating barriers to wide-scale XFSC implementation we expect that the toolkit will not only accelerate therapeutic translation, but also to reduce long-term costs, address ethical concerns, promote reproducibility, and take the necessary steps to move toward truly autologous personalized medicine.

F-2136

DISSECTING THE CELLULAR RESPONSES OF IMMUNE CELLS AND LGR5+ STEM CELLS IN THE INFLAMED MOUSE COLON BY SINGLE-MOLECULE RNA ISH

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Due to its exposure to a harsh luminal environment, the epithelium of the adult mammalian intestine has a remarkably fast turnover rate that is facilitated by a resident intestinal stem cell (ISC) population present at the base of the intestinal crypt. These ISCs, marked by the GPCR Lgr5, allow the intestinal epithelium to adapt to different types of damage, such as inflammation. Chronic intestinal inflammation is a hallmark of the inflammatory bowel diseases (IBD), and intestinal organoids generated from adult ISCs are a promising treatment for IBD, yet the interplay between inflammatory immune cells and ISCs remains to be elucidated. The intestinal

crypt is a well-defined structure in cellular orientation and differentiation, making it an ideal model system to study the cellular responses of immune and stem cells during inflammation. Therefore, we utilized the single-molecule RNA in situ hybridization (ISH) assay RNAscope to visualize the expression of multiple immune and ISC markers within the inflamed intestinal tissue environment. This assay is a well-suited method for the cellular resolution of resident ISCs and their progenitors in the normal and inflamed intestine because it detects single RNA molecules in individual cells with morphological context. To interrogate the expression pattern of inflammatory immune cell and ISC markers within the intestinal crypt, we performed the assay on colons from either control or TNBS-treated mice. We visualized the location of each intestinal cell population, including the resident Lgr5+ ISC population, within the crypt. The impact of inflammation on the Lgr5+ ISC population, as well as the Wnt/β-catenin pathway, was also examined. Using a multiplex assay, we assessed and quantified the ratio of regulatory T cells and Th17 cells in the inflamed region. Lastly, we examined the expression of several receptor-ligand pairs for cytokines and ISC markers. Taken together, these results demonstrate the ability of the RNAscope assay to visualize the ISCs within the morphological context of the intestinal crypt and in relationship to inflammatory immune cells. The use of such an assay can aid in elucidating the direct effects of inflammatory cues on the ISCs and their niche during the pathogenesis of IBD and other inflammatory diseases, as well as developing potential therapeutics.

F-2138

ESTABLISH FLUORESCENT PROTEIN REPORTER SYSTEMS FOR THE GENERATION OF COMPLETELY REPROGRAMMED BOVINE INDUCED PLURIPOTENT STEM CELLS

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The induced pluripotent stem cells (iPSCs) have been successfully established across species from mice to humans. iPSCs also hold great promise in improving livestock animal reproduction and disease resistance/biomedical disease modeling. However, the generation of bona fide bovine iPSCs (biPSCs) has been difficult. There are three main issues associated with established biPSCs: 1) Reliance on exogenous transgenes for self-renewal, 2) Limited replication capacity, and 3) Limited differentiation potential. These indicate incomplete reprogramming of the biPSCs. Fluorescent reporters controlled by pluripotency marker genes have greatly facilitated the screening of reprogramming conditions and the identification of completely reprogrammed mouse and human iPSCs, as well as the generation of naïve pluripotent human iPSCs/ESCs from the primed state. We hypothesize that the fluorescent protein reporters controlled by bovine pluripotency marker will facilitate the generation and isolation of bona fide biPSCs. To this end, we established lentiviral vector based green and red fluorescence protein (GFP/RFP) reporter system controlled by bovine Oct4 and Nanog gene promoters, respectively. We demonstrated the activity of these fluorescent reporter systems in mouse and human embryonic stem cells and induced pluripotent stem cells. Although these reporters show no activity in differentiated somatic cells, fluorescent signals could be detected at two to three weeks after mouse somatic cell reprogramming, which is the period when cells gradually becoming completely reprogrammed. The generation of bovine specific fluorescent reporters will help identify new reprogramming paradigms for the successful establishment of bona fide pluripotent biPSCs.

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F-2140

IMPACT OF PASSAGING METHOD ON IPSC QUALITY DURING EARLY CLONAL ESTABLISHMENT

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Sendai viral-based reprogramming has emerged as the gold standard for the generation of induced pluripotent stem cells (iPSC) from diverse somatic cell sources including hard-to-manipulate blood-derived cells. As iPSC progress towards translational application, there is a need to establish best practices that ensure consistent quality. This requires robust and streamlined methods for clonal establishment of iPSC that are viable, pluripotent, and foot-print free. In this study, iPSC were generated from both fibroblasts and blood-derived cells using the Sendai-virus based CytoTune 2.0 Reprogramming kit in Essential 8 media system. At least 5 clones respectively from each reprogramming were isolated and subjected to either traditional manual passaging or bulk passaging with EDTA. Cell survival and morphology for each clone was monitored up to passage 10. Additionally, RNA was collected at alternate passages to monitor viral clearance. Clones passaged manually showed better survival and morphology compared to bulk passaged clones. Further, majority of clones were footprint-free, albeit the manual passaged clones had faster rate of

viral clearance. On average, manually passaged clones also had better survival and morphology during clonal expansion. These results indicate manual passaging to be a better method for early clonal establishment. Given the tedious nature of this method, alternate approaches are being currently explored that are less labor-intensive that produce high quality iPSC clones. This is important for rapid, large-scale generation of iPSC from diverse sources.

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F-2142

OPTOGENETICALLY ENGINEERED HUMAN NEURONAL PRECURSORS FOR TRANSPLANTATION IN THE NORMAL AND INJURED RODENT BRAIN

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Optogenetically engineered human neuronal precursors (hNPs) have great potential as tools in regenerative neuroscience: their fate can be tracked in vivo by their fluorescence reporter gene (survival and differentiation) and the functionality of their differentiated neuronal progenies can be assessed as response to light stimulation or inhibition. In experiments we present here, we engineered H9 embryonic stem cell-derived hNPs with pLenti-hSyn-hChR2-eYFP-WPRE virus. NP differentiation strategy utilized a protocol optimized for cortical neuron fate. Neuronal precursors were transplanted into the brain (motor cortex) of normal rodents and subjects injured via impact acceleration. To assess fate specificity and functionality of the optogene, ChR2-eYFP (+) NPs were first differentiated to cortical neurons in vitro and shown to generate action potentials and response to light stimulation. Transplanted NPs survived well and, 2 months after transplantation, 70 % of such cells were seen to have differentiated into neurons with axons extending over the corpus callosum to reach certain remote targets, such as piriform cortex. We are presently exploring the degree of synaptic maturation of neurons derived from hChR2-NP transplants, their structural integration with the host nervous system, and their optogenetic functionality in vivo. We propose that optogenetically engineered hNPs hold great promise as tools to explore the repair of the injured central nervous system with stem cell strategies.

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F-2144

A FULLY AUTOMATED "SMART" SYSTEM: PROOF OF CONCEPT OF IN-PROCESS MONITORING IN CELL THERAPY MANUFACTURING BY 10 PASSAGE COMPARATIVE STUDY OF HIPSC EXPANSION

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Conventional cell therapy manufacturing is performed by manual operations, and quality is usually controlled by testing of final products. However, manufacture of high quality cell therapy products with improved yield and lower production costs requires the next generation of cell therapy manufacturing – a fully automated "smart" system. This enables consistent high quality production from highly variable raw materials and processes, with non-invasive in-process monitoring providing quality control during manufacture. We performed a proof of concept study of automated cell therapy manufacturing and in-process quality monitoring through in-depth analyses during a 10 passage culture of CGT-RCiB10, a research-grade hiPSC established by Cell and Gene Therapy Catapult from a GMP pre-seed lot. Both manual and automated cultures using Essential-8/Vitronectin-N/Accutase were completed. At each passage, daily microscope imaging and analysis of spent medium (pH, DO, DCO2, glucose, lactic acid, glutamine, glutamic acid, and 95 metabolite panels by liquid chromatography-tandem mass spectrometry (LC-MS/MS)) were performed. At P0, P5 and P10, detailed cellular characterization, including gene expression profiling by TaqMan®Scorecard, cell surface marker and transcription factor analysis by flow cytometry, karyotyping (G-banding and CGH) and directed differentiation assays, were completed. Multivariate analysis was performed and results indicate that 1) cells grown in the automated system for 10 passages maintained pluripotency, high viability, normal karyotype, and three germ layer differentiation potential, 2) highly reproducible and more consistent data was obtained by automated culture compared to manual

culture, 3) metabolic and cell growth data suggests there is increased oxygen availability in the automated culture plate due to its unique design, and 4) image features from phase contrast microscopy have strong correlation with the metabolic profile of cells . Based on this learning, Tokyo Electron is currently developing GMP-compliant automated systems equipped with in-process monitoring which enable scale-up production and differentiation of clinical grade hPSCs.

F-2146

SMALL-MOLECULE COCKTAIL FOR RAPID MATURATION OF HUMAN NEURONS

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A major application of neurons derived from human induced pluripotent stem cells (iPSCs) is to model neurological or psychiatric diseases for use as a drug discovery platform. Most phenotypes of neurological and psychiatric diseases arise in mature neurons. However, human iPSCs-derived neurons can take 1-3 months to reach full functional maturation, and yet manipulating the neuronal cultures for even 2 weeks in 384-well plates is cumbersome. Therefore, the substantial time required for achieving maturation is a severe hurdle for taking full advantage of human neurons as drug discovery platforms. To overcome this hurdle, we have engineered a human iPSC reporter line with a fusion of nanoluciferase (Nluc, Promega) with synaptophysin (SYP), a synaptic vesicle glycoprotein that is expressed in virtually all mature neurons and acts as a marker for quantification of synapses. By screening with the SYP-Nluc reporter, several compounds were identified that accelerate SYP expression. After further optimizing the combination and concentrations of these compounds, we have developed a neuronal maturation cocktail. The cocktail can be applied to multiple neuronal types, including spinal motor neurons, cortical glutamatergic neurons, and cortical GABAergic neurons. After treating with the cocktail, the neurons displayed extensive neurite outgrowth within 3 days, expressed pre- and post-synaptic mature markers within 7 days, and exhibited electrophysiological activity within 2 weeks. This rapid maturation cocktail will enhance the ability to model CNS disease processes and screen new drugs.

Funding Source: NIDA/NIH SBIR Grant 1R43DA042659-01 to Zhong-Wei Du

F-2148

DERIVATION OF CLINICALLY COMPLIANT GMP HUMAN EMBRYONIC STEM CELL LINES UNDER DEFINED AND XENO-FREE CONDITIONS

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Cell transplantation has a great potential as treatment for many diseases. Human embryonic stem cells (hESC) can differentiate in vitro to many different cell types and are therefore a suitable source for cell therapies. However, there is a lack of high-quality hESC derived under GMP conditions, which is crucial for clinical translation. We now present our efforts in generating new clinically compliant GMP hESC lines and our optimized protocol for defined and xeno-free GMP production. Although we have previous published xeno-free and defined protocols for derivation and culture several of the culture components did not hold GMP standards when scrutinized in detail. We have now re-evaluated all the components in the original protocol resulting in a new media formulation and GMP compatible laminin521 culture matrix. All reagents have subsequently been functionally tested to allow high clonal growth and ultimately colony formation from blastocyst outgrowths. With GMP protocols in place, equipped GMP laboratory and ethical approval from the Regional Ethics Board in Stockholm we are now initiating the first derivations within Vecura, a GMP facility within Karolinska University hospital using surplus human embryos, donated with informed consent. We hope these cells will be soon available to the research community as starting material for stem cell-based regenerative medicine.

F-2150

EXTRACELLULAR VESICLES FROM FETAL BOVINE SERUM AND HUMAN PLATELET LYSATE MODIFY MESENCHYMAL STROMAL CELL GROWTH

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Recently, a lot of attention has focused on the potential applications of extracellular vesicles (EVs), defined as 50-150 nm membrane-bound particles containing biomolecules including RNAs. For example, EVs can reproduce the response generated by stem cells to

injury and disease, and, recent data suggests that microRNAs inside EVs mediates their signaling. Fetal Bovine Serum (FBS) and Human Platelet lysate (HPL) are used routinely as a medium supplement for the expansion of mesenchymal stromal cells (MSCs). Currently, it is unknown whether EVs isolated from FBS or HPL can duplicate this growth and attachment effect. Here, we compared EVs isolated from FBS or HPL to determine whether they support MSC attachment and proliferation. MSC were grown in DMEM + FSB or + HPL as the control conditions and EVs isolated from FBS or HPL were used to determine whether they can support MSC attachment and proliferation. Proliferation was determined by AO/PI staining and cell counting and by MTT assay. Since EVs are non-antigenic and non-infectious, they have potential as a medium supplement for good manufacturing process of MSCs.

F-2152

A NOVEL METHOD FOR EFFICIENT AND PRECISE HUMAN GENOME EDITING IN HUMAN INDUCED PLURIPOTENT STEM CELL

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Requirements of precise and efficient genome editing are dramatically increasing. Current technologies using Zinc finger proteins (ZFN), Transcription activator-like effector nucleases (TALEN) and clustered, regularly interspaced, short, palindromic repeats (CRISPR)/Cas systems are utilized to develop mutant mice and human disease models, faster than traditional gene targeting methods, but with limitations, such as off-site effects, time-consuming and labor-intensive. To facilitate the genome editing in mammalian system, we design a novel concept for genome editing method. The c.35delG and c.109G>A mutations in the GJB2 gene have been identified as important genetic mutations of hearing impairment as they accounted for the majority of mutations in deafness. We demonstrated that c.35delG and c.109G>A mutations could be created in the GJB2 genomic locus of human induced pluripotent stem cells (iPSCs) without interference with the expression levels of endogenous GJB2 and pluripotent genes, such as Oct4 and Sox2. Our novel method will facilitate precision and efficient genome editing not only for basic science but also for clinical and regenerative medicine.

Funding Source: BETA Biosystem Co., LTD

F-2154

EFFICIENT DERIVATION OF HUMAN EMBRYONIC STEM CELLS IN CLINICAL-GRADE MEDIUM AND MATRIX

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Human embryonic stem cell lines are currently being used in clinical trials for treatment of various diseases including diabetes and retinal pigment epithelial disorders. The majority of human ES cell lines have been derived using mouse fibroblast feeders and media containing animal-derived products, and most ES cells are not autologous to the patient. The efficient derivation of human ES cell lines in defined, xeno-free conditions could enable the derivation of autologous ES cell lines by nuclear transfer in GMP conditions. Here we show that the medium STEM FIT in combination with a recombinant laminin 511E8 matrix enables efficient feeder-free and xeno-free derivation of human ES cell lines from IVF blastocysts, which are pluripotent and karyotypically stable. This provides a path to the derivation of GMP grade personalized ES cells.

F-2156

MESENCHYMAL STEM CELL-DERIVED OSTEOBLAST CULTURE DYNAMICS REVEALED BY HIGH THROUGHPUT SINGLE CELL TRANSCRIPTOME- WIDE PROFILING

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The plasticity of mesenchymal stem cells (MSCs) to differentiate into multiple cell types provides a promising future of regenerative medicine in heart, cartilage, and bone repair. Pluripotent MSCs can be initiated and directed into various lineage commitments using chemical factors and even physical factors, such mechanical forces in the extracellular matrix both in vitro and in vivo. Because MSCs are highly malleable, even individual MSCs within the same culture may have different responses and differentiation patterns to generate a heterogeneous population. However, how these individual MSCs respond to differentiation cues and how their gene expression differs remain to be addressed. To better understand stem cell heterogeneity, we employed a high throughput single cell transcriptome-wide RNA-seq approach using BDTM Resolve system to profile ~10,000 single MSCs committed to the osteogenic lineage at various time points using a commercially-defined differentiation

protocol. As expected, we found that MSC cultures driven to the osteogenic lineage are mosaic, with both pluripotency and osteogenic genes to be expressed at highly variable levels. Nonetheless, we were able to identify gene sets that are enriched along with pluripotent and osteogenic markers, providing a basis of understanding MSC differentiation dynamics. This assay can be utilized in other stem cell culture systems to enhance research efforts in stem cell biology and regenerative medicine.

F-2158

THE EUROPEAN BANK FOR INDUCED PLURIPOTENT STEM CELLS (EBISC) - AN INTERNATIONAL SOURCE OF QUALITY-CONTROLLED, DISEASE-RELEVANT, RESEARCH-GRADE HUMAN IPSC LINES

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The European Bank for Induced Pluripotent Stem Cells (EBiSC) is a not-for-profit iPSC bank providing researchers across academia and industry with access to quality-controlled, disease-relevant, research-grade iPSC lines. The public catalogue (https://cells.ebisc.org) aims to be the largest European catalogue of human iPSC lines by making available cell lines generated by multiple institutes from one centralized resource. Although European focused, EBiSC ships iPSC lines worldwide, with sales and distribution managed by the European Collection of Authenticated Cell Cultures (ECACC). Our role at EMBL-EBI is as one of a number of partners developing the EBiSC Information Management System, which facilitates the complete journey of a cell line, from depositor to purchaser via banking at EBiSC's core facility. Depositors register cell line information via hPSCreg (http://hpscreg.eu), a mandatory registration system for European generators of iPSCs for describing and annotating their cell lines. Deposited cell lines are banked and expanded at a central facility with standardized protocols and quality assessment. The portal (https://cells.ebisc.org) acts as a central database to coordinate interaction between the federated services, and to provide a public portal for customers to browse the EBiSC catalogue and follow direct links to purchase lines. Cell line associated genetic data are distributed via the European Genome-phenome Archive (EGA; https://www.ebi.ac.uk/ega), a secure managed-access archive specializing in sharing patient-linked data with bona fide researchers. Alongside powerful metadata searches we are currently developing the capability to search lines based on their underlying genetic variation data, enabling querying by specific mutations or particular alleles which will importantly include managed access lines for which the user has an access agreement in place. The IMS makes available all iPSC-associated protocols (generation, characterization, expansion and differentiation) and employs ontologies to standardize annotations and structure searches. The implementation of community standards complements EBiSC's wider commitment to driving forward standards in the quality control, characterization, and distribution of European cell lines.

Funding Source: EBiSC has received support from the IMI Joint Undertaking (grant 115582), resources of which are composed of financial contribution from the EU's 7th Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.

F-2160

A FLUORESCENCE-BASED SCREENING PLATFORM FOR MODULATION OF CRISPR/CAS9-STIMULATED HOMOLOGY DIRECTED REPAIR OF POINT-MUTATIONS

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Recently, we have described an improved in cella platform for the evaluation of homology directed repair (HDR) stimulation by Cas9-sgRNAs and double nickases at defined target loci of choice. This double nickase approach was successfully used for targeted, mono- or bi-allelic correction of point-mutations in patient-derived iPSC by both, selectable long flanking arm donors as well as ssODNs. However, global and unselected HDR efficiencies were in the range of 0.5-1% even for the most suitable sgRNA/double nick combinations. Potential in vivo applications of the CRISPR/Cas9-system for stimulation of HDR demand for higher efficiencies in order to genetically correct a therapeutically relevant number of cells. Here, we demonstrate a novel fluorescence-based screening platform for analysis of small molecule compounds or genetic elements, which could influence HDR. Importantly, the system is based on a single (A/G) point-mutation which completely ablates fluorescence of the fluorophore and which can efficiently be corrected by a proximal sgRNA. This platform allowed us to efficiently analyze various types of DNA donors or DNA damages for their HDR-stimulation efficiencies. Moreover, we also evaluated a number of previously proposed chemical and biological stimulants of HDR. Intriguingly, the small molecule-compound L755507 suppressed HDR in HEK293T-reporter cells, which stands in contrast to the HDR-enhancement effect reported earlier for this substance, whereas Brefeldin A had no effect compared to DMSO control. Currently, we are adapting the system

for use in other cell lines and cell types. Together, our point-mutation-based HDR reporter-system allows for fast and accurate evaluation of HDR efficiencies in various cell lines and under various conditions.

F-2162

DEVELOPING A MULTIPLEX MICROSCALE SCREENING PLATFORM BY MULTI-PHOTON BIOFABRICATION TECHNOLOGY FOR MAINTENANCE OF CELL PHENOTYPE

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Multiphoton biofabrication is an emerging technology that makes use of photochemical crosslinking to immobilize natural biological molecules with sub-micron spatial resolution without involving harsh reagents and fabrication conditions. Our group previously demonstrated the capability of the technology in constructing micro-patterns with various controllable mechanical properties and extracellular matrix (ECM) immobilization. Moreover, we have successfully made use of the ultra-high spatial resolution to construct various topological features including both 2D and 3D ones such as pillar array, convex, concave, cave and niche. The excellent controllability makes the technology a pertinent platform for screening purposes. The microscale fabrication technique also enables the investigation of primary cells or other less-readily available cell types. This study aims to develop such a platform to screen for the optimal microenvironment for phenotype maintenance of cells types that transform spontaneously during in vitro culture, like spontaneous differentiation of mesenchymal stem cells and the dedifferentiation of nucleus pulposus cells (bNPCs). This study investigates the effect of mechanical properties (elastic modulus, stiffness), ECM composition (fibrinogen, fibronectin, laminin, vitronectin, collagen type I) and topological features (both 2D and 3D) in phenotype maintenance. Micropatterns were fabricated by a femto-second laser, in the presence of photosensitizer. The marker expressions were evaluated and quantified by immunofluorescent staining and intensity quantification after certain days of culture. The effect of different factors was examined and scored. Take bNPC as an example. High level of fibrinogen was found to favor the expression of collagen type II (one of the bNPC markers), while the effects of fibronectin were less significant. Low modulus and stiffness were also demonstrated to favor the phenotype maintenance.

Funding Source: The Research Grants Council (NSFC-RGC N HKU713/14)

F-2164

DISSOLVABLE MICROCARRIERS FOR HMSC AND HIPSC PRODUCTION AND RECOVERY

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A new dissolvable microcarrier technology was developed that supports efficient cell production and recovery while eliminating the need for microcarrier separation. Dissolvable microcarriers are made from calcium crosslinked polygalacturonic acid polymers that are easily dissolved using a solution of EDTA and pectinase. To enable cell adhesion in serum-containing and serum-free applications, microcarriers are pre-coated with either porcine-derived denatured Collagen or Corning® Synthemax™ II, a synthetic vitronectin peptide polymer. We demonstrate human mesenchymal stem cell (hMSC) and human induced pluripotent stem cell (hiPSC) growth on dissolvable microcarriers in spinner flasks and bioreactors. Upon microcarrier dissolution, nearly 100% of cells were recovered, and cells maintained their respective phenotype and differentiation capability.

F-2166

EFFICIENT, FOOTPRINT-FREE GENE EDITING OF IPS CELLS FOR CLINICAL APPLICATIONS USING CRISPR/CAS9

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The combination of two powerful technologies, human induced pluripotent stem (hiPS) cells and precise, footprint-free editing using CRISPR/Cas9, allows for a new level of sophistication in development of disease models. The ability to create hiPS cell lines from donors with disease specific-mutations and to edit mutations into specific backgrounds will enable discoveries with a new level of granularity. Despite progress in improving nuclease specificity and reducing off-target activity with precise tools like CRISPR/Cas9, a major challenge for successful gene editing in hiPSCs is the lack of culture systems that allow researchers to isolate single hiPSCs with the desired mutations and to generate stable, healthy, clonal lines from edited cells. Traditionally, hiPS

cells are grown and passaged as colonies. In order to obtain single cells for cloning, the colonies must first be dissociated into a single-cell suspension, which often results in cell death or premature differentiation. Furthermore, gene editing protocols often subject stem cells to harsh conditions that compromise their health and survival. Using the DEF-CS-500 culture system, we can culture hiPS cells in a monolayer with a very high rate of single-cell survival and clone expansion. We used this culture system to develop a complete workflow, starting with CRISPR/Cas9-mediated editing, using Cas9/sgRNA ribonucleoprotein (RNP) complexes delivered into hiPS cells via either electroporation or cell-derived nanoparticles called gesicles, followed by successful single-cell cloning of edited hiPS cells. We chose non-DNA-based delivery methods to guarantee footprint-free editing of the hiPS cells. We achieved endogenous gene knockout efficiencies of up to 65% for the membrane protein CD81 in a hiPS cell population. We also achieved efficient, accurate knock-in using electroporation with long ssDNA donor fragments. We demonstrated that edited hiPS clones obtained with this workflow were still pluripotent and have a normal karyotype, even after further expansion. The data show this workflow using footprint-free editing via efficient delivery of Cas9/sgRNA RNP complexes and single-cell cloning of hiPS cells in modified media, results in a high number of edited and expandable hiPS clones that maintain the hallmarks of pluripotency.

F-2168

GENETIC MODIFICATION OF HUMAN PLURIPOTENT STEM CELLS USING A DOUBLE OLIGONUCLEOTIDE CRISPR/CAS RECOMBINATION SYSTEM

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Pluripotent stem cells (PSCs) are valuable tools for the study of human biology and in vitro disease modeling. The ability to introduce and/or correct disease-associated mutations using the CRISPR/Cas9 system promotes greater tractability within these specialized cells. However, engineering of highly specific alterations has been challenging, prompting the development of new methodologies to enhance the efficiency and ease of genome engineering in stem cells. A limiting factor in genome editing includes generating precise heterozygous modifications. To overcome this obstacle, we demonstrate the use of a double oligonucleotide (ODN) recombination system to introduce and/or correct mono-allelic genetic mutations. When using a single-stranded ODN (ssODN) to generate a heterozygous mutation, we found a high incidence of indel occurrence in the unaffected allele. To overcome this problem, we introduced two ssODNs, both of which contain the necessary blocking mutations to prevent gRNA recognition and re-cutting, but only one of which includes the desired genetic alteration. This method introduced heterozygous alterations at an efficiency of 8-21% in multiple genes and pluripotent cell lines, without occurrence of indel formation. This new methodology provides a streamlined approach for generating valuable tools to model human disease in stem cells.

F-2170

FULLY INTEGRATED CLOSED SYSTEM EXPANSION AND DIFFERENTIATION OF PLURIPOTENT STEM CELLS TOWARDS MESENCEPHALIC DOPAMINERGIC PROGENITOR CELLS

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Pluripotent stem cell (PSC) derived cell products hold great promise for future clinical use in a variety of indications like TypeI diabetes, cardiomyopathies, macular dystrophies or Parkinson's disease. Raising regulatory requirements for such advanced-therapy medicinal products (ATMPs) imply the need for standardized reagents and highly reproducible production procedures. Automation of PSC expansion, differentiation, and potentially product optimization through cell sorting may contribute to successful and cost-effective innovative therapies. Using our versatile and integrated GMP cell processing platform CliniMACS Prodigy® we previously developed a cultivation and expansion workflow for iPS cells. Now we have translated the differentiation to mesencephalic dopaminergic (mesDA) progenitor cells to the device. Adapting this protocol from an embryonic body based to a fully adherent differentiation paradigm enabled straight forward upscaling of a lab protocol to a medium-scale production process within the closed system. One million PSCs were used as starting material for expansion in the Lam521-coated Centrifugation and Cultivation Unit (CCU). An in-process control revealed a pluripotent marker profile and a 30 to 60 fold expansion in the first cultivation phase. After 11 days of differentiation in a Lam111-coated CellStack (636cm2), we harvested around 800 million mesDA progenitor cells, before cells were passaged for final

differentiation until d16. Extrapolating the cell numbers retrieved from the protoype process would correspond to 200-250 patient doses assuming 5 million cells per cryopreseved unit. However, the process holds further potential for upscaling. To characterize the identity of the resulting d16 progenitors we have designed a concise marker panel for flow cytometry based quality control (QC). Taken together, we have developed a method for adherent, closed-system cultivation of PSCs and differentiation to dopaminergic progenitor cells in combination with comprehensive QC assays.

Funding Source: This study was supported by grants from the European Community's 7th Framework Programme through NeuroStemcellRepair (#602278)

F-2172

A NOVEL ELECTROPORATION DEVICE FOR THE HIGHLY EFFICIENT, VERY LOW CYCOTOXIC TRANSFECTION AND GENOME EDITING OF HUMAN STEM CELLS

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Transfection plays an important role in manipulating human stem cells for research and clinical applications. Unfortunately many types of human stem cells, including ES/iPS cells and primary human stem cells are generally considered "hard-to-transfect" for their resistance to common methods introducing exogenous nucleic acids. Development of novel non-viral, pure physical methods which does not require any additional potentially hazardous reagents for stem cell transfection is highly desirable. We systematically analyzed the defects of traditional electroporation method, and designed a novel device that avoids these defects, to achieve the highly homogeneous electric field distribution within the electroporation vial. Our results showed that the new device allowed us to achieve very high efficiency in transfecting various types of human stem cells, including human ES/iPS cells (74.0%), neural stem cells (83.1%) and mesenchymal stem cells (78.2%). Cytotoxicity, which is generally high with electroporation method, is greatly reduced and for many cell types is completed avoided. When the method was applied to deliver proteins and siRNAs into the cells, nearly 100% efficiency was achieved for all the cell types tested. We then tested the method for genome editing in human stem cells using the CRISPR/Cas9 technique. We showed that CRISPR/Cas9 elements can be efficiently delivered into human stem cells. Notably, we observed a very high homologous recombination (HR) rate (40%) at specific sites, and a high consistency among the efficiencies of the same gRNA in different cell types. These results suggest that the previously reported low HR rate, and the inconsistency of gRNA efficiency in different cell types may be attributed to the insufficient delivery of CRISPR/Cas9 elements into the cells. Our device provides a novel powerful tool for these studies, and may greatly facilitate the clinical application of human stem cells.

WEDNESDAY, JUNE 14, 2017

LATE BREAKING POSTER SESSION I-ODD 18:30 – 19:30

W-3001

EPHB2 SIGNALING-MEDIATED SIRT3 EXPRESSION RESCUES HUMAN UMBILICAL CORD BLOOD-DERIVED MSCS SENESCENCE VIA MNSOD EXPRESSION AND MITOCHONDRIAL ROS HOMEOSTASIS

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Though expansion of human umbilical cord blood derived-mesenchymal stem cells (UCB-MSCs) in vitro is inevitable for clinical use, their limitation of replicative capacity is an obstacle in challenging UCB-MSCs for regenerative medicine. Disruption of mitochondrial reactive oxygen species (mtROS) homeostasis is a key factor of inducing UCB-MSCs senescence, and consequently developing the approach of preventing mtROS accumulation will help to prolong the conversion into senescent state of UCB-MSCs. In this study, we observed that the expression of EphrinB2 and its receptor, EphB2, was regulated reversely during serial culture expansion of UCB-MSCs. Activation of EphB2 by treatment with EphrinB2 dose-dependently decreased the SA-β-gal activity and expression of p16 and p21, cellular senescence markers. EphrinB2 increased the expression and mitochondrial translocation of Sirt3, a major mitochondria NAD+-dependent deacetylase. Knock down of Sirt3 by siRNA transfection inhibited the effect of EphrinB2-reduced senescence of UCB-MSCs. EphrinB2 lead to nuclear translocation of Nrf2, and Sirt3 expression was regulated by Nrf2 transcripional activity dependent manner. Among the Sirt3 target genes, EphrinB2 treatment increased the expression of MnSOD, an mtROS scavenger, and reduced mtROS level of UCB-MSCs. Furthermore, EphrinB2-induced Sirt3 increased MnSOD activity by deacetylation at lysine 68

residue. In conclusion, EphrinB2-induced suppression of senescence progression is promoted by MnSOD-mediated mtROS scavenging through EphB2-Nrf2-Sirt3 signaling pathway in UCB-MSCs. These results indicate that EphB2-mediated signaling has a crucial role in maintaining replicative ability of UCB-MSCs and EphB2 receptor can be a novel marker for optimization of therapeutic use of UCB-MSCs in regenerative medicine.

W-3003

AGE AND ANATOMICAL LOCALIZATION OF MOUSE BONE MARROW-RESIDENT ADIPOGENIC PROGENITOR CELLS DETERMINE ADIPOCYTE PHENOTYPE

Ambrosi, Thomas H., Graja, Antonia, Jank, Anne-Marie and Schulz, Tim
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Aging and diet-induced obesity promote the expansion of an adipocytic lineage in the bone. Accumulation of fat cells impairs the two major regenerative processes of this organ, e.g. osteogenesis and hematopoiesis. In contrast, expansion of marrow fat during caloric restriction has been shown to significantly contribute to circulating Adiponectin, thereby supporting a healthy metabolic profile. These data suggest functionally distinct types of marrow adipose tissue (MAT), as observed in the traditional fat depots. Although it is known that MAT can be classified into distinct constitutive and regulated fat cells, its relationship to the regular depots of white and brown fat is still a matter of discussion. In long bones, MAT traces to a subcutaneous white adipocyte-like developmental origin. Fat cells derived from isolated adipogenic progenitors (APCs) of femur and tibia show comparable brown-specific marker expression (Ucp1, Cidea) to cells from white adipose tissue and muscle under basal conditions and are non-respondent to a browning-stimulus by bone morphogenic protein (BMP)7. Microarray analysis further uncovers similarities in progenitors independent of their source tissue: APCs from young, but not aged, mice express high levels of extracellular matrix-associated genes suggesting a role in local tissue remodeling which might be impaired with age. Comparative gene expression analyses of different bones reveals similar levels of white fat markers, but increased transcript levels of brown fat markers specifically in sternal bone samples that decline during aging. In accordance, and unlike other bones, adipogenic progenitor cells from the sternum are highly responsive to BMP7-mediated white-to-beige adipocyte conversion. Taken together, these findings suggest that bone compartments with increased hematopoietic activity, such as the sternum, may harbor aging sensitive brown-like adipocytes, whereas the peripheral skeleton contains fat cells that more closely resemble a white adipogenic phenotype.

W-3005

MIMICKING EMBRYOLOGICAL VASCULAR DEVELOPMENT IN LIPOSOME-LADEN HYDROGELS

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Vascularization of tissue constructs is one of the critical challenges preventing the advancement of tissue engineering to clinical applications. Thus, in the past decade, there has been a rapid rise in the derivation of functional microvascular blood vessels from pluripotent stem cells (PSCs) both in vitro and in murine models, with potential applications as therapies for cardiovascular diseases and as models for vascular development. Many of these platforms utilize endothelial cells that have been fully differentiated prior to seeding in biomaterials. However, without the addition of pericytes, these PSC-derived endothelial cells will often form unstable, leaky lumen. Here, we propose a controlled-release, hyaluronic acid-based hydrogel to regulate the differentiation of vascular progenitor cells (VPCs) to endothelial cells and pericytes, thereby creating stabilized, three-dimensional vasculature. The biphasic release of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) from phospholipid liposomes simulates the growth factor profiles observed in embryological development, and more importantly, prevents the formation of the PDGF-Rβ/VEGF-R2 complex, which has been shown to block pericyte recruitment in the later stages of angiogenesis. We describe the synthesis of injectable hydrogels from hyaluronic acid with the controlled release of VEGF and PDGF and show that VPCs remain viable after rapid crosslinking at 37°C. The measured elastic modulus of the hydrogels is approximately 4 kPa, which closely mimics the stiffness of the native endothelial microenvironment. We also demonstrate the derivation of mature CD31+VECad+ endothelial cells and α-SMA+ pericytes from induced PSCs of different lineages by utilizing a cost-effective, small molecule approach.

Funding Source: American Heart Association (15SDG25740035), National Institute of Health T32 Ruth L. Kirschstein Fellowship (EB007507)

W-3007

CD11A IDENTIFIES PRECURSORS TO HEMATOPOIETIC STEM CELLS IN THE MOUSE EMBRYO VIA NEONATAL TRANSPLANTATION

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Hematopoietic stem cells (HSCs) are the multipotent and self-renewing progenitors of the blood system with the ability to reconstitute the blood system of a recipient upon transplantation. Production of HSCs from induced pluripotent stem cells (iPSCs) promises a limitless source of patient-specific donor HSCs. This could potentially eliminate immune response-induced compilations of transplantation therapy. Generation of iPSC-derived HSCs has been unsuccessful, as these cells lack bone marrow (BM) engraftment potential and resemble an immature stage in HSC development. Similarly, the precursors to HSCs in the embryo, "pre-HSCs", are multipotent and self-renewable but lack BM engraftment potential. The ability to FACS-purify pre-HSCs will allow deeper characterization of the molecular differences between immature pre-HSCs and fully-functional HSCs, therefore addressing the gaps in our understanding of how HSCs naturally arise during embryonic development. This information can then be applied to the generation of functional iPSC-derived HSCs. We have determined the efficiency of CD11a (part of LFA1 receptor) for purification of murine adult HSCs. Also, our previous results established that only the CD11a- fraction of embryonic hematopoietic progenitors contains multipotent potential in vitro. To functionally assess putative pre-HSCs in vivo, we utilized a neonatal transplant system. This system bypasses the immediate BM homing/engraftment requirement as the neonatal liver can offer a more accessible site of hematopoiesis for maturation and eventual BM homing of pre-HSCs. We tested the efficacy of CD11a as a pre-HSC marker in a competitive setting and at embryonic stages prior to the emergence of proliferative fully-functional HSCs. Our data reveals that only the CD11a- fraction of progenitors (defined as Ter119- CD43+ Sca-1+ Kit+ CD144+) at embryonic days 10-11.5 shows long-term engraftment post-transplantation, suggesting the enrichment of pre-HSCs within this population. The combination of these markers allows for the identification and isolation of a rare population of pre-HSCs as well-defined as adult HSCs. Furthermore, utilization of CD11a as a marker of pre-HSCs has allowed us to identify candidate genes whose expression can potentially improve BM homing/engraftment of pre-HSCs.

W-3009

OFF-THE-SHELF HEMATOPOIETIC PROGENITOR CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR THE TREATMENT OF AUTOIMMUNE AND INFLAMMATORY DISEASES

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CD34+ hematopoietic progenitor cells (HPCs) play a key role in immune-surveillance. We and others have demonstrated that HPCs traffic to sites of inflammation and inhibit disease causing T-cells through immune-checkpoint pathways including PD-L1 and IDO1. Importantly, we show that HPCs suppress T cell activation and effector function independently of HLA matching. These properties suggest HPCs might serve as an effective "off-the-shelf" cell therapy for the treatment of autoimmune and inflammatory diseases. To overcome some of the limitations associated with using patient-derived HPCs, including limited scalability and costly manufacturing, we have established a manufacturing process to generate "off-the-shelf" immunoregulatory iHPCs from induced pluripotent stem cells (iPSCs). This GMP-compatible, feeder-free differentiation protocol is highly scalable, enabling production of over 1,000 iHPCs from each iPSC. iHPCs produced with this process are highly enriched for myeloid markers (>95% purity) and are free of granulocytes, erythrocytes, and lymphoid cells. In co-culture assays, iHPCs are potent suppressors of TCR-driven T cell proliferation and cytolytic activity. We are currently exploring the efficacy and persistence of iHPCs in a xenogeneic mouse model of Graft-versus-Host-Disease (GvHD). Together, our results demonstrate that iHPCs may serve as a scalable, "off-the-shelf" source of immunoregulatory cells for the treatment of autoimmune and inflammatory diseases.

Funding Source: Corporate funding

W-3011

TRACING THE EMERGENCE OF HEMATOPOIETIC STEM CELLS FROM THE EXTRA-EMBRYONIC YOLK SAC IN MICE

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While iPSCs offer the potential for an unlimited source of patient HSCs, the generation of transplantable HSCs from iPSCs has yet to be achieved. This suggests that a better understanding of how HSCs arise naturally in the course of embryonic development is needed. The first primitive waves of hematopoiesis arise from the embryonic yolk sac (YS), but definitive waves arise later and produce the first HSCs. Which tissues produce definitive hematopoiesis, and thereby HSCs, remains unclear. The aorta-gonad-mesonephros (AGM), placenta, YS, and vitelline vessels are all potential sources. Using a Lyve1-Cre lineage tracing reporter, we previously published with Dr. Hanna Mikkola's lab that Lyve1-Cre marks definitive hematopoietic cells that arise primarily in the YS, with some labeling in the vitelline vessels, and show that about one-third of adult HSCs are derived from Lyve1-expressing precursors. Our central hypothesis is that the extra-embryonic YS is a significant source of adult HSCs. We have found that Lyve1-derived cells contribute to each definitive wave of embryonic hematopoiesis. Lyve1-marked pre-HSCs from the early embryo are transplantable and give rise to adult HSCs. Lyve1-derived adult HSCs contribute to cells in all of the major blood lineages. Interestingly, there seems to be a bias in the development of lymphoid cells, suggesting that Lyve1-derived HSCs have functional properties distinct from HSCs that arise from other tissues. Taken together, this data suggests that Lyve1-derived cells give rise to engraftable, multipotent, and long term self-renewing HSCs. We interpret these results to support the notion that the extra-embryonic YS is an HSC nice in the embryo and contributes to adult hematopoiesis.

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W-3015

INVESTIGATING THE ROLE OF METABOLISM IN SKIN HOMEOSTASIS AND TUMOR INITIATION BY HAIR FOLLICLE STEM CELLS

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For an increasing number of cancers, the cell of origin has been demonstrated to be the resident adult stem cell. One such cancer is squamous cell carcinoma (SCC), for which recent studies in our lab traced its origin to the hair follicle stem cells (HFSCs). While many studies have demonstrated the intrinsic and extrinsic mechanisms that regulate the cycling nature of these stem cells, less clear is how quiescence versus activation alters their metabolism, and whether the metabolic state of HFSCs contribute to both the maintenance of tissue homeostasis and the initiation of tumors. Malignant transformation is thought to coincide with a dramatic shift towards the use of glycolysis and establishment of a 'Warburg' state – increased metabolism of glucose to lactate. How the Warburg Effect is established during tumor initiation in vivo remains unclear. The current consensus is that the bulk of the energy generated in most adult tissue cells is created by oxidative phosphorylation, while more highly proliferative cells, such as activated immune cells and cells transformed to make a tumor, mainly use glycolysis. Little is known about how individual cell types generate energy in vivo, however, and how their metabolism influences basic cell fate decisions such as cell division, migration or differentiation. Using genetically engineered mouse models that allow the study of both tissue homeostasis and the Warburg Effect in vivo, I have made important observations that provide the basis for new investigations into the role of metabolism in key cell fate decisions by adult stem cells. My findings indicate that HFSCs possess a unique metabolic profile that may be critical for their maintenance and for their response to oncogenic insults. Importantly, they suggest the possibility that the "Warburg Effect" is the result of the expansion of an already glycolytic subpopulation, namely the HFSCs. I hypothesize that the glycolytic state of hair follicle stem cells regulates their quiescence and makes them more vulnerable to transformation. If in fact the unique metabolism of these stem cells predisposes or makes them more vulnerable to transformation, the possibility of shifting their metabolism away from glycolysis to reduce or eliminate their ability to give rise to squamous cell carcinomas will be the subject of intense investigation.

Funding Source: UCLA Eli and Edythe Broad Stem Cell Research Center

W-3017

A NOVEL METHOD TO CULTURE HUMAN AMNIOTIC STEM CELLS WITH DELICATE GROWTH PATTERNS FOR CLINICAL APPLICATION

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Human amniotic stem cells are a valuable source of cells for cell therapy. Scientists are putting efforts to figure out good ways to culture them in xeno-free media for clinical application. The initial nich may be the best environment for stem cell growth . Here we use umbilical cord blood serum from the same willing donor in place of FCS or serum substitute supplement to culture the amniotic stem cells. Both the hAECs and hAMCs cultured in umbilical cord blood serum express all the major pluripotent genes Sox-2, Oct-4 and Nanog as well as maitaining the expression of the embryonic stem cell specific surface antigens SSEA-4, TRA-1-60 and TRA-1-81 up to 5 passages. Using the appropriate differentiation medium, these amniotic stem cells were shown to differentiate into adipocytes, osteocytes, chondrocytes and neuronal cells, as visualized by specific staining. Especially, immunophenotype analysis of hAECs and hAMCs show an delicate growth patterns with wonderful aesthetic values under cultured in umbilical cord blood serum. Thus, we can conclude that, human amniotic stem cells could maintain their stem cell charateristics when cultured in the umbilical cord blood serum. It should be possible a novel culture method for stem cells to improve their stemness and differentiation potentials for future clinical applications.

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W-3019

NEURAL DIFFERENTIATION CAPACITY OF HUMAN MESENCHYMAL STEM CELLS FROM DIFFERENT SOURCES: A COMPARATIVE STUDY

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Neural stem cells (NSC) are solely located in certain regions of the brain. Accordingly, the option of NSC to repair damaged regions of the brain becomes limited. The above could be the case of Parkinson Disease and Amyotrophic Lateral Sclerosis. Several preclinical and clinical studies have demonstrated that Mesenchymal Stem Cells (MSC), which are present in many adult tissues, including bone marrow, fat tissue, umbilical cord and skin, can be differentiated into neural progenitor cells. By using MSC isolated and ex vivo expanded from the above indicated tissues, we investigated their capability to differentiate to neural phenotypes expressing: early (Nestin, β-III tubuline) structural (NEFM, NEFL) and functional markers (Synaptophysine, post synaptic protein 90, Nuclear receptor related 1 and Thyroxine hydroxylase). The results of these studies indicate that adipose tissue-derived MSC exhibit: a) the highest expression of both structural and functional markers, b) a higher proliferative capability, and c) important changes from fibroblastoid to neural morphology. Accordingly, these results suggest that adipose tissue-derived MSC represents an interesting cellular source of reparative cell therapies for neural diseases.

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W-3021

REGENERATIVE CAPACITY OF ADULT MOUSE BRAIN

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Cell death in the mouse adult brain is observed among neurons and glial, microglial and blood vessel cells. At the same time, the brain has been shown to have an intrinsic ability to replace these lost cells. In our study we evaluated the regenerative capacity of the adult mouse brain and how it changes in the mouse from 1 month old to 2.5 years old. We developed a technique that allows us to count and map the location of all proliferating cells in the entire brain of adult mice. Next we identified neurogenic proliferative zones in

the mouse brain using doublecortin immunostaining. We found that neurogenesis occurs at two locations in the mouse brain. The larger area we define as the main proliferative zone (MPZ). This zone includes the lateral walls of the lateral ventricles, often called subventricular zone. The smaller one corresponds to the subgranular zone (SGZ) in the dentate gyrus of the hippocampus. Next we classified proliferating cells into 3 categories based on their location in the MPZ, SGZ and the parts of the brain outside of neurogenic zones. We found that the number of proliferating cells in the neurogenic zones progressively decreases as the mouse ages. The extent of this decrease is about 15-times in the MPZ and even greater in the SGZ where it is about 64-times. Previously it was shown that with an increase in age neuronal losses increase. At the same time, the brain's ability to produce new neurons appears to be decreasing. One might speculate that this misbalance could be a contributing factor for a cognitive decline in aged mice. Unlike neural progenitors, the number of cells that proliferate outside of neurogenic zones remains fairly stable from the age of 2 months to the age of 2.5 years showing that the rate of replacement of non-neural cells such as cells of blood vessels and microglia remains at the same level during the entire mouse lifespan. In summary, our data show that the activities of stem cells in the MPZ, SGZ and areas outside of neurogenic zones change differently as the mouse ages indicating that their age-dependent activity might be regulated by different mechanisms.

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W-3023

INTRANASAL DELIVERY OF hUCB-MSC REVEALS A NOVAL EPOXYEICOSATRIENOIC ACID SECRETOME PREVENTING CHOLESTEROL ACCUMULATION AND RESCUE IMPAIRED AUTOPHAGY IN NIEMANN PICK TYPE C1 DISEASE

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Niemann pick type C1 (NPC1) disease is a neurological disorder in which cholesterol and gangliosides accumulate in late endosomes/ lysosomes, followed by rapid death of purkinje neurons. Our previous data have demonstrated that human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) have the multifunctional abilities to ameliorate NPC1 symptoms of NPC1 transgenic mice model. Given that safety and efficacy of cell-based therapies for neurodegenerative diseases depends on the mode of cell administration, direct transplantation of cells to the CNS can be invasive and precluded to apply in the clinic. Therefore, the present study examined the therapeutic efficacy of intranasal delivered hUCB-MSC using NPC mice. After nasal delivery motor function and purkinje cell survival improved while GFP expressing hUCB-MSCs were detected mainly in the olfactory bulb, rostral migratory stream, and only single cells were observed in the hippocampus and cerebellum. We concluded the therapeutic effect of hUCB-MSC was mediated by secreted paracrine factors rather than cell-to-cell interaction. Through this study we evaluated hUCB-MSC to be a secretion source for 14,15-EET, demonstrating 14,15-EET as a therapeutic factor for NPC1 patients. In in vitro study, 14,15-EET reduced cholesterol accumulation in human NPC1 patient fibroblast and ameliorating autophagic signals and impaired cholesterol synthesis. Taken together, intranasal administrated hUCB-MSC provides highly encouraging alternative to the traumatic surgical transplantation and highlights a promising potential therapeutic strategy for NPC1 patients by presenting 14,15-EET as a novel candidate for a therapeutic factor.

Funding Source: This research was supported by the Research Institute for Veterinary Science, Seoul National University (SNU, Republic of Korea).

W-3027

HIGH-RESOLUTION MICROELECTRODE ARRAY PLATFORM FOR BIOMARKER DISCOVERY AND NOVEL FUNCTIONAL ASSAYS IN HUMAN IPSC-DERIVED NEURONS

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Microelectrode-array (MEA) technology enables label-free and high-throughput recording of cellular electrical signals. MEAs are currently used for in vitro phenotype characterization and drug toxicity/efficacy testing with iPSC-derived cells. Here, we present a high-resolution microelectronics-based MEA featuring 26'400 microelectrodes within a sensing area of 3.85 mm × 2.10 mm. The electrical activity of whole cell networks can be monitored and studied at high spatio-temporal resolution. The electrical activity of individual cells can be isolated and investigated, together with subcellular features, such as the propagation speed of action potentials along single axons. The high-resolution MEA system can extract novel parameters from iPSC-derived cells, which can be used as potential biomarkers for phenotype screening and drug testing. We performed novel functional assays in iPSC-derived midbrain-floorplate dopaminergic neurons. We analyzed the electrical phenotype of an A53T α-synuclein

dopaminergic neural line, modeling Parkinson's disease. We found differences in physiological activity between the A53T α-synuclein cell line and the isogenic control cell line. Phenotype differences were detected at different scales, ranging from network activity to subcellular structures as axons.

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W-3029

EFFECTS OF OXIDIZED LOW DENSITY LIPOPROTEIN ON PROLIFERATION OR DIFFERENTIATION OF MOUSE NEURAL PROGENITOR CELLS INTO NEURAL CELLS

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[Introduction] Although previous reports showed that high fat diet feeding induced anxiety, depression, or cognitive dysfunction via inhibition of mouse hippocampal neurogenesis, the direct effects of dyslipidemia on neurogenesis are unknown. The present study determined involvement of oxidized low density lipoprotein (ox-LDL) on proliferation or differentiation of mouse neural progenitor cells. [Methods] Mouse neural progenitor cells were derived from the embryoid bodies of mouse induced pluripotent stem (iPS) cells stimulated with Noggin. The proliferation of neural progenitor cells was determined by MTT assay. The neural differentiation was induced by stimulation with all trans retinoic acid (ATRA; 3 microM). The differentiation potential from neural progenitor cells into neural cells was evaluated by NeuN expression using immunofluorescence staining or western blot analysis. [Results] Although the treatment with ox-LDL did not affect the proliferation of mouse neural progenitor cells, the treatment with ox-LDL (10 microM) significantly inhibited ATRA-induced NeuN expression. We have also shown that mouse neural progenitor cells express ox-LDL receptor 1 (OLR-1). Pretreatment with OLR-1 siRNA significantly blocked the inhibitory effect of ox-LDL. In addition, treatment with ox-LDL significantly reduced the phosphorylation of both Akt and CREB. [Conclusion] These results suggest that the stimulation with OLR-1 inhibits the differentiation of mouse neural progenitor cells into neural cells via inhibition of Akt and CREB.

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W-3031

VASCULARIZED TISSUE-ENGINEERED MODEL FOR STUDYING DRUG RESISTANCE IN NEUROBLASTOMA

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Neuroblastoma is a vascularized pediatric tumor derived from neural crest stem cells that can express a number of stemness markers, including SOX2 associated with the undifferentiated stem cell phenotype. Recently, expression of SOX2 was demonstrated in a number of cancers and related to drug-resistance and tumor relapse. However, the role of SOX2 in neuroblastoma progression remains poorly understood. We engineered a perfusable, vascularized in vitro model of three-dimensional human neuroblastoma to study the effects of retinoid therapy on tumor vasculature and drug-resistance. The bioengineered model recapitulated the clinically observed vasculogenesis and vasculogenic mimicry, and contained subpopulations of stem-like neuroblastoma cells expressing high levels of SOX2. Treatment with Isotretinoin augmented populations of cells expressing high levels of SOX2 without targeting new blood vessels formed from SOX2+/CD31+ cells. Our results reveal some roles of SOX2 in drug resistance and tumor relapse, and suggest that SOX2 could be a therapeutic target.

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W-3033

EPOP/C17ORF96 INTERACTS WITH ELONGIN BC AND USP7 TO MODULATE THE CHROMATIN LANDSCAPE IN MOUSE EMBRYONIC STEM CELLS

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Gene regulatory networks are pivotal for many biological processes. In mouse embryonic stem cells (mESCs), the transcriptional network can be divided into three functionally distinct modules: Polycomb, Core, and Myc. The Polycomb module represses developmental genes, while the Myc module is associated with proliferative functions, and its mis-regulation is linked to cancer development. Here, we show that, in mESCs, the Polycomb repressive complex 2 (PRC2)-associated protein EPOP (Elongin BC and Polycomb Repressive Complex 2-associated protein; a.k.a. C17orf96, esPRC2p48, and E130012A19Rik) co-localizes at chromatin with members of the Myc and Polycomb module. EPOP interacts with the transcription elongation factor Elongin BC and the H2B deubiquitinase USP7 to modulate transcriptional processes in mESCs similar to MYC. EPOP is commonly upregulated in human cancer, and its loss impairs the proliferation of several human cancer cell lines. Our findings establish EPOP as a transcriptional modulator, which impacts both Polycomb and active gene transcription in mammalian cells.

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W-3037

HIGH-FIDELITY DRUG INDUCED LIVER INJURY SCREEN USING HUMAN IPSC LIVER ORGANOIDS

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Prediction of potential drug induced liver injury (DILI) remains a major challenge in drug development. Here, we developed a human liver organoid model derived from pluripotent stem cells applied for modeling human DILI pathology. We directly differentiated human iPSC into posterior gut endoderm, followed by self-organized generation of polarized human liver organoids (pHLO) under defined conditions. The pHLO contains human polarized hepatocytes and an internal lumen lined by these cells, recapitulating the in vivo bile canaliculi-like architecture. Dynamic visualization confirmed the unidirectional uptake of fluorescent bile acid (BA) analogue, and subsequent excretion of BA into the lumen of pHLOs. By leveraging this micro-anatomical feature, we developed a relatively simple and versatile human DILI model. In the presence of 9 FDA-approved drugs including potential DILI drugs, organoid based live screen not only confirmed decreases in cell viability in vitro but enabled a multi-parametric mechanistic classification of individual drugs based on mitochondrial toxicity and cholestasis. Furthermore, we extended this approach towards vulnerability in cells, demonstrating pHLO under lipotoxic conditions promoted severe DILI. Interestingly, lethal liver damage can be alleviated with N-acetylcysteine. To our knowledge, this is the first human liver organoid based model for analyzing DILI, facilitating lead compound optimization and mechanistic study, as well as anti-DILI therapy screening applications.

Funding Source: Japanese Science & Technology, Ono Pharmaceutical, The New York Stem Cell Foundation

W-3039

CELL AND NICHE ENGINEERING TO ENHANCE HUMAN PLURIPOTENT STEM CELL - DERIVED HEMATOPOIESIS

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Human pluripotent stem cells (hPSCs) provide an exciting cell source for regenerative therapy because they propagate indefinitely while maintaining their potential to differentiate towards specialized tissue types. However, in vitro generation of hPSC-derived specific cell types, such as definitive hematopoietic stem cells (HSCs), remain challenging due to the complex interplay between spatial and temporal parameters during HSC generation. Here, we tackle these challenges by a combined reverse- and forward engineering approach. The reverse engineering approach involves the recapitulation of developmental hematopoiesis using developmental niche engineering. Specifically, using microfabricated micropatterns, hPSC-derived haemogenic endothelial (HE) cells were formulated into spatially organized, size-controlled colonies and the effect of endogenous signaling on blood cell yields was measured. Size-specific haemogenic niches displayed 5.5-fold enhanced blood cell induction over non-micropatterned cultures. The platform was next used

to identify the mechanism behind this enhancement and IP-10 was discovered as an inhibitor for hPSC-derived CD45+ blood cells; IP-10 inhibition could be rescued by anti-CXCR3 antibody treatment and p38 MAPK inhibition implicating a role for the p38 MAPK signaling pathway during hematopoietic emergence. In parallel, we are applying this platform to a synthetic biology forward engineering approach that takes advantage of synthetic gene networks using a modular design framework termed bow-tie. The framework is composed of a module that specifically detects the presence or absence of multiple hPSC blood progenitor cell microRNA, and in response controls a fluorescent reporter (or selectable element) using RNAi-based logic design rules. The microRNA profile and network design can be computationally programed from microRNA Seq data of defined blood progenitor cell types from human cord blood. Upon stable integration of this network into hPSC, the lines are being combined with our engineered niches to detect desired blood progenitor cell types and to systematically test and dose small molecules to skew the differentiation towards the desired cell fate.

W-3041

THE PATENT APPLICATION TREND OF INDUCED PLURIPOTENT STEM CELL (IPSC) TECHNOLOGIES

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Objective: To investigate patent application trend of disease-specific iPSC technologies. Methods: Using PATENTSCOPE (WIPO database), we extracted unexamined patent publication bulletins (hereinafter called "publications") that describe iPSCs in the claims. In the disease-specific iPSC technologies, we reviewed the technical elements and the target disease described in the claims. The technical elements of disease-specific iPSC invention were categorized into genome editing iPSCs, patient-derived iPSCs, disease-specific drug screening method, and diagnosis method. Results: The total number of publications that claimed iPSCs was 1424, of which 726 publications (about 54% of the total number of publications) were filed by the applicants of the US nationality, 376 publications (about 26%) were filed by the applicants of Japanese nationality. The number of the publications related to iPSC manufacturing method, differentiation induction method, and culture method was 204, 353, and 161, respectively. Regarding the disease-specific iPSC technologies, on the other hand, there were 79 publications (about 5% of the total number of publications), of which 49 publications were filed by the applicants of the US nationality, 15 publications were filed by the applicants of Japanese nationality. The number of the publications related to genome editing iPSC technology, patient-derived iPSC technology, disease-specific drug screening method, and diagnosis method were 43, 10, 16, and 10, respectively. In classification of the target disease group, the number of publications was in order of neuromuscular disease group (21 publications), blood disorder group (15 publications), and mental disorder group (11 publications). The main target disease was muscular dystrophy and amyotrophic lateral sclerosis in the neuromuscular disease group, hemoglobinopathy in the blood disease group, and Alzheimer's disease in the mental disorder. Conclusions: Almost of the inventions of iPSC technologies are filed by the applicants of the US nationality and Japanese nationality. A bank of disease-specific iPS cell is actively building. Therefore, for freedom to research/operate, it will be necessary to identify the patents covering the banked disease-specific iPSC as well as the scope of the patented claims.

W-3043

HMGB1 ADMINISTRATION AMELIORATES CUTANEOUS AND NON-CUTANEOUS MANIFESTATIONS IN THE MOUSE MODEL FOR AN INHERITED CONNECTIVE TISSUE DISEASE, DYSTROPHIC EPIDERMOLYSIS BULLOSA

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High mobility group box 1 (HMGB1) is a non-histone chromatin protein which controls transcription by regulating chromatin structure. During non-physiological events, such as a necrotic condition, HMGB1 is known to be released into the circulation and plays pivotal roles in tissue regeneration and immunological processes. Previously, we have demonstrated that the released HMGB1 mobilizes platelet-derived growth factor receptor alpha (PDGFRα) positive bone marrow cells into the damaged tissues using SDF-1α/CXCR4 axis. We have also shown the multi-lineage differentiation capacity of the mobilized PDGFRα positive bone marrow cells; the cells can differentiate into both fibroblasts and keratinocytes, and provide type VII collagen (Col7) at the cutaneous basement membrane zone. Based on these results, we asked if the systemic administration of HMGB1 can become a promising therapeutic approach for moderate forms of dystrophic epidermolysis

bullosa (DEB), which possess amino acid substitution mutations in at least one allele of Col7a1 gene to produce less-functional anchoring fibrils and induce dermal-epidermal separation. To test this hypothesis, we systemically administered HMGB1 to DEB model mice. The treatment with HMGB1 significantly improved the survival with restoring the expression of Col7 at the basement membrane of the skin and the mucosa, while most of the untreated control mice died within 20 weeks due to severe malnutrition. Correlated with the recovery of Col7 expression, the DEB symptoms were ameliorated. Together, these data position HMGB1 as a promising drug at least for moderate forms of DEB. Furthermore, our study provides a proof-of-concept for a treatment in which the multipotent cells from bone marrow are mobilized to damaged tissues, as a promising therapy for diseases of the cutaneous, and possibly non-cutaneous tissues with severe intractable damages, such as DEB.

W-3045

THE UNIQUE FEATURE OF H3K9ME3-DEPENDENT HETEROCHROMATIN REPROGRAMMING AND LANDSCAPE REBUILDING IN EARLY EMBRYO

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H3K9me3-dependent heterochromatin is thought to reprogram during fertilization to reactive highly specialized paternal and maternal genome to totipotency. This repressive modification will further involve in embryonic gene expression silence as well as imprinted X inactivation during development and is build. However the molecular details are lacked due to limited material. Here in this study we mapped the genome-wide profiles of H3K9me3 from matured germ cell to early implant embryo and systematically analyzed the remover and rebuild feature of mutable histone markers. We found H3K9me3 exhibited unique feature in both fertilization and cell fate determination compared to H3K4me3 and H3K27me3, and even itself showed district dynamic change in promoter and classical H3K9me3-dependent heterochromatin regions. We also found the rebuild of H3K9me3 helped on the transcription regulation of LTR retro-transposons during embryo development and further function in lineage determination during implantation.

W-3047

SIRT2 IS REQUIRED FOR EFFICIENT REPROGRAMMING OF MOUSE EMBRYONIC FIBROBLASTS TO PLURIPOTENCY

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The roles of sirtuins (SIRTs) in cancer biology have been the focus of recent research. Based on the similarity between underlying pathways involved in the induction of pluripotent stem cells (iPSCs) and transformation of cancer cells, the role of SIRTs in pluripotency reprogramming has been revealed. In mammals, seven SIRTs have been identified; SIRT2, has a controversial role in cancer, and has been classified as both a tumor suppressor and promoter. Herein, we sought to elucidate its role in iPSC reprogramming. It was found that absolute deprivation of SIRT2 in mouse embryonic fibroblasts (MEFs) resulted in a notable reduction in reprogramming efficiency. SIRT2 depletion not only upregulated elements of the INK4/ARF locus, which in turn had an anti-proliferative effect, but also significantly altered the expression of proteins related to the PI3K/Akt and Hippo pathways, important signaling pathways for stemness. This study thus indicates that SIRT2 positively affects reprogramming, and highlights a putative role of SIRT2 as a tumor promoter.

W-3049

EXPLORING THE ROLE OF THE LNCRNA XACT IN HUMAN X CHROMOSOME INACTIVATION

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X-chromosome inactivation (XCI) is essential to equalize the dosage of X-linked gene products between females and males in mammals. Failure to properly silence one of the two X-chromosomes during female embryonic development is lethal. Once established on one X, the inactive state is highly stable. However, in human embryonic stem cells (hESCs) and certain pathological contexts including cancer, the inactive X shows sporadic reactivation. Thus, dedicated mechanisms have to be engaged to ensure the proper developmental timing and the stability of XCI. In placental mammals, the X-linked lncRNA XIST initiates a cascade of events in cis that leads to the silencing of the entire chromosome. Whereas XIST holds the central role, recent studies have highlighted remarkable differences between species in the molecular control of XCI and XIST regulation

during development and differentiation. Our group has identified XACT, a human-specific lncRNA which accumulates on active X chromosomes in human early embryonic contexts. XACT expression is mostly restricted to pluripotent cells and it is repressed upon differentiation. Here we present our most recent work on the identification of human-specific mechanisms regulating X chromosome activity during early development. Imaging and single-cell RNA-sequencing reveal that XIST and XACT are both expressed and accumulate on active X chromosomes in both early human pre-implantation embryos and in naïve hESCs. In these contexts, the XIST RNA shows a highly-dispersed distribution, which might be linked to its inability to induce silencing of the X chromosome. Furthermore, forcing the expression of XACT in a mouse heterologous system impairs the accumulation of Xist during mouse ES cell differentiation. We are now undertaking a series of mechanistic and functional studies to understand the role of XACT in X chromosome activity and its regulation during early human development and in naïve hESCs.

W-3051

COMPARISON OF METHODS FOR HUMAN IPSC HAEMATOPOIETIC DIFFERENTIATION TO STUDY ABERRANT HAEMATOPOIESIS

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Direct comparison between induced pluripotent stem cell (iPSC) haematopoietic differentiation methods is essential for informed selection of the optimum method for a particular endpoint. Yet despite over 100 publications and increasing numbers of new methods patented each year, there are virtually no published comparisons of methods. Cellular output is an important consideration for minimising cost and time required to produce sufficient cells for downstream analysis. Our aim herein is to compare four methods adapted from published literature which are free of feeder layers and serum: two embryoid body and two monolayer methods, each with simple versus multistep differentiation, utilising various iPSC lines and independent replicates. The monolayer multistep differentiation (2D multistep) method resulted in higher CD34+ progenitor cell production, both in purity (mean 31±3% SEM, $p < 0.0001$ ANOVA) and absolute numbers (460,000±96,000 CD34+ cells derived from 106 iPSC, $p < 0.0001$), and enriched numbers of functional haematopoietic progenitors (8,034±1838 colony forming units (CFU) per 106 iPSC, p=0.0002). One of the greatest utilities of iPSC is to recapitulate the haematopoiesis of the patient from whom iPSC were derived. All four methods were able to detect aberrant haematopoiesis, recapitulating the phenotypes of Down Syndrome (DS) and β-thalassemia. Importantly, only the 2D multistep method showed the expected increased frequency of total DS-derived CFU (Wild type (WT) 9,649±3,038 versus DS 57,360±14,750 CFU per 106 CD34+ cells, p=0.0029). The 2D multistep method gave rise to increased frequency of megakaryocyte progenitors (CFU-Mk) from DS-derived iPSC (WT 62,600±13,450 versus DS 136,900±15,510 CFU-Mk derived from 106 CD34+ cells, p=0.0065). β-thalassemia (β-thal) derived iPSC showed decreased total CFU (WT 17,460±4,678 versus β-thal 1,685±610, p=0.0212) and BFU-E (WT 9,064±2,973 versus β-thal 200±154 CFU per 106 CD34+ cells, p=0.0341), consistent with previous reports. Haemoglobin gene expression by real time PCR showed all methods express similar amounts of γ-globin, with minimal detectable β-globin. In conclusion, the 2D multistep method resulted in the greatest cellular output, with utility to recapitulate different forms of aberrant haematopoiesis from patient-derived iPSC.

W-3053

HUMAN PLURIPOTENT STEM CELL-DERIVED THYMIC EPITHELIAL PROGENITOR CELLS AS STEM CELL-BASED THERAPY TO RESTORE THYMIC FUNCTION IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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Hematopoietic stem cell transplantation (HSCT) remains the only curative therapy for many malignant and non-malignant diseases. HSCT outcome and recipient thymic function are intimately intertwined, and the thymus plays a key role in post-transplant immune reconstitution. In fact, the production of mature T cells occurs in the thymus and depends on the interaction of hematopoietic progenitor cells with non-hematopoietic stromal elements, notably thymic epithelial cells (TECs). Through positive and negative selection of maturing T lymphocytes on TECs, the thymus generates a broad and self-tolerant T cell receptor (TCR) repertoire that protects from infection and prevents autoimmunity. At odds with its importance for successful transplant outcomes, the thymus is exquisitely sensitive to a wide range of insults encountered during the transplant

period, including cytotoxic agents and irradiation, as well as acute graft versus host disease (GVHD). Thymic injury in turn, leads to susceptibility to infections, autoimmunity and chronic GVHD, the leading causes of non-relapse related mortality and morbidity. Thymic function naturally declines with age with no endogenous potential to regenerate. Current treatments options for thymic injury during HSCT are limited to symptomatic therapy. Therefore, novel approaches to cure irreversible thymic injury are critically needed. By recapitulating developmental cues and lineage bifurcations that occur during thymic ontogeny, our goal is to generate fully functional and mature thymic epithelial progenitor cells (TEPCs) from iPSCs as a stem cell-based therapy for thymic regeneration and restoration of immune competence in HSCT recipients. Here we show that TEPCs derived in vitro from hPSCs express the key thymic transcription factor FOXN1 along with Keratin 5 and Keratin 8, the primary cytoskeletal building blocks of medullary and cortical TECs, respectively. To validate the fidelity of our in vitro platform, we are comparing the transcriptome of hPSCs-derived TEPCs with that of primary medullary and cortical thymic epithelial cells. Our ongoing work is testing the capacity of hPSCs-derived TEPCs to promote the development of a broad and self-tolerant TCR repertoire in a humanized mouse model.

Funding Source: Stanford School of Medicine, DiGeorge Syndrome Project

W-3055

GENERATION OF hiPSC-DERIVED HUMAN PERIPHERAL SENSORY NEURONS RELEASING SUBSTANCE P ELICITED BY ANANDAMIDE

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Neural Crest Stem Cells (NCPCs) have been shown to differentiate into various cell types and tissues during embryonic development, sensory neurons included. There have been few studies addressing the generation of NCPCs and peripheral sensory neurons (PSNs) from human induced Pluripotent Stem Cells (hiPSCs). However, most studies failed to show robust activity of the PSNs generated. These cells would be powerful tools to investigate somatosensory biology and pathology and its interaction with Human Epidermal Keratinocytes (HEK) to study skin physiology and inflammatory response, besides screening for novel antinociceptive drugs/analgesics. Here, we describe an efficient lineage-specific protocol for differentiation of hiPSCs into NCPCs and PSNs. hiPSCs were exposed to chemically defined media under feeder-free conditions and three small-molecule compounds to achieve efficient differentiation into lineage-specific NCPCs in 10 days, confirmed by the expression of specific markers. After completed NCPC differentiation, the cells were maintained in neural induction medium containing defined growth factors for peripheral sensory neurons differentiation, followed by ten days of treatment with conditioned medium from HEK. We observed a further increase in the expression of PSN differentiation markers such as TRPV1 and Islet-1 after conditioned medium treatment. The neurons generated were functional in response to anandamide, which triggered the release of substance P (SP). Calcium signals were modest in response to capsaicin and other agents. In conclusion, this alternative protocol allows high yield of NCPCs and the use of PSNs for biotechnological applications and cell therapy on a higher scale. Moreover, this protocol can be applied to patient-derived hiPSCs and thus serve as a powerful tool to model human diseases for drug discovery.

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W-3059

SMALL MOLECULE ATF6 ACTIVATOR PROMOTE MESODERMAL DIFFERENTIATION OF HESC/HIPSCS

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ATF6 encodes a transcription factor that is activated during the Unfolded Protein Response (UPR) by Golgi endoproteolysis to protect cells from ER stress. Loss of ATF6α and its closely related paralog ATF6β, results in early embryonic lethality in mouse, defective notochord formation in fish, and in people, loss of ATF6α specifically, results in abnormal retina formation that leads to severe congenital vision loss manifesting in infancy. These developmental phenotypes implicate an essential role for ATF6 in vertebrate development. We investigated the function of ATF6 in human stem cells

as they differentiate into multi-potent germ layers and subsequent nascent tissue types. We artificially activated ATF6 during stem cell differentiation using a recently identified small molecule selective ATF6 agonist, and we inhibited ATF6 using iPSCs generated from patients harboring ATF6 mutations. We discovered that ATF6 suppresses pluripotency, enhances differentiation, and surprisingly, guides differentiating human stem cells toward mesodermal cell fates. Our findings reveal a novel role for ATF6 in human stem cell differentiation and identify a new strategy to robustly create functional tissues of mesodermal origin through modulation of the ATF6 arm of the UPR.

W-3061

CALMODULIN INHIBITORS RESCUE THE EFFECTS OF RIBOSOMAL PROTEIN DEFICIENCY BY MODULATING P53

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Ribosomal protein (RP) mutations are found in many diseases, including Diamond Blackfan anemia (DBA), where defective erythropoiesis, craniofacial abnormalities and increased cancer risk are major complications. RP mutations cause p53 activation through accumulation of free RPs that bind and sequester MDM2, the negative regulator of p53. p53 knockout in models of DBA have shown that p53 mediates many phenotypes of DBA. To find new therapies for DBA our lab generated induced pluripotent stem cells (iPSCs) from DBA patients with mutations in RPS19 and RPL5. These cells recapitulate DBA phenotypes; they have increased p53 activity and a reduced capacity to differentiate into red blood cells (RBCs). To overcome their limited proliferative capacity, we respecificed the DBA iPSCs into expandable CD34+ (CD34-5F) progenitors with five previously identified transcription factors. We used these DBA mutant CD34-5F cells to perform a chemical screen for rescue of erythroid differentiation. Out of the eleven top hits from the screen, two were calmodulin (CaM) inhibitors. Interestingly, we also found CaM inhibitor hits in a separate in vivo chemical screen performed in a DBA zebrafish model. Injection of the FDA-approved CaM inhibitor, trifluoperazine (TFP), into a DBA inducible mouse model significantly increased hemoglobin levels and reduced p53 activity in the bone marrow. Further investigation of TFP mechanism demonstrated that TFP normalized p53 to wildtype protein levels. Decreased p53 protein was due to TFP inhibiting the accumulation of p53. It is known that regulation by the 5' and 3' UTR of p53 can increase p53 translation during stress and DNA damage. Using a luciferase construct flanked by 5' and 3' UTR of p53, TFP treatment significantly reduced the luciferase signal compared to DMSO treated. This data suggests that TFP counteracts the increased translation of p53, leading to less p53 and less apoptosis of RBC precursors. In conclusion, we have shown that CaM inhibitors identified in two different chemical screens are able to improve erythroid differentiation in multiple in vitro and in vivo models of DBA through the reduction of RP-deficient induction of p53. Our data strongly suggests that CaM inhibitors may be effective therapies for DBA patients, and a clinical trial is being planned with TFP for 2017.

W-3063

PATIENT-SPECIFIC iPSC-DERIVED HEPATOCYTE-LIKE CELLS GENERATED FROM ARCHIVED PBMC SAMPLES AS A MODEL OF PHENPROCOUMON-INDUCED LIVER INJURY

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The reprogramming of somatic human cells to induced pluripotent stem cells offers great possibilities for regenerative medicine as well as for disease modeling and drug screening purposes. This is due to the capability of iPS cells to be both expanded virtually indefinitely and differentiated towards any cell type possible, including cell types relevant for certain diseases. The directed differentiation towards hepatocyte-like cells is of importance for drug screenings, since the liver serves as the major drug-metabolising organ in the body. Additionally, it can be particularly useful for modeling of diseases directly affecting the liver. In this study, we first established a facilitated protocol for generating patient-specific hepatocyte-like cells for use in disease modeling and drug screening. Peripheral blood mononuclear cells represent one of the easiest accessible cell sources and can be readily stored. We used small numbers of archived PBMCs as a starting cell source and successfully generated iPS cells by transduction of polycistronic lentiviral vectors encoding codon-optimised cDNAs of OCT4, SOX2, KLF4, and C-MYC. In the derived iPS cell

clones, pluripotency characteristics, like expression of SSEA4, TRA1-60, OCT4, SOX2 and NANOG, could be observed. The differentiation potential of the iPS cells towards all three embryonic germ layers was examined in vitro using the Scorecard Assay. Also, their amenability to hepatic in vitro differentiation was confirmed by analysing the expression of hepatic marker genes, such as ALB, HNF4α, AFP and TTR, as well as the capability to secrete albumin. We then used hepatically differentiated cells derived from a phenprocoumon-induced liver injury patient in an attempt to model the cytotoxic effect of the drug on the cells in vitro. With this, we established a protocol for obtaining patient-specific iPS cells from minimal numbers of easy-access blood cells by making use of frozen PBMC libraries. Upon directed differentiation towards hepatocyte-like cells, these iPSCs can now be used for drug screening as well as modeling of liver diseases, such as the phenprocoumon-induced liver injury investigated in this study.

W-3065

CHANGES IN H3K27ME3 ASSOCIATED WITH WOUND HEALING PROPERTIES IN IPSC-DERIVED DIABETIC FOOT ULCER FIBROBLASTS

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Diabetic patients exhibit poor wound healing that leads to debilitating complications such as diabetic foot ulcers (DFUs). Fibroblasts are critical to normal wound healing but show abnormal functions in DFUs. However, it is not known how epigenetic changes in diabetic primary fibroblasts lead to poor wound healing outcomes or if they can be reversed. Repressive H3K27me3 mark is a one of the major histone marks that changes during reprogramming and differentiation. We have reprogrammed both normal and diabetic fibroblasts into induced pluripotent stem cells (iPSCs) and have subsequently differentiated them into fibroblasts, with the goal of understanding the epigenetic profile linked to improved wound healing. Our goal is determine how epigenetic changes can impact wound healing after reprogramming of fibroblasts to iPSCs and subsequent differentiation to fibroblasts. Previously, we identified that diabetic primary ulcer-derived fibroblasts (DFUFs) deposit thin extracellular matrix (ECM) rich in Type I Collagen and Fibronectin in 3D in vitro self-assembled tissues that mimic the connective tissue seen in chronic wounds. In addition, DFUFs are limited in inducing angiogenic response and secret decreased levels of pro-angiogenic cytokines CXCL12, IL6, and CCL2. We analyzed whether genes involved in phenotypes associated with chronic wound healing are being regulated by H3K27me3. We performed ChIP-qPCR analysis and identified that DFUFs had greater H3K27me3 levels in COL1A1 and FN promoters compared to NFFs and iPSC-derived fibroblasts. H3K27me3 was increased in DFUFs and iPSC-derived fibroblasts in CXCL12 and IL6 promoters. We found that CCL2 and Serpine 1 promoters showed increased levels of H3K27me3 in DFUFs that were erased by reprogramming to iPSC and subsequent differentiation. We also determined that total levels of H3K27me3 protein were lower in diabetic primary fibroblasts compared to normal primary fibroblasts. Overall, we observed the differential presence of H3K27me3 mark in the genes involved in chronic wound healing by DFUFs and identified their modulation upon reprogramming and differentiation.

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W-3069

MODELING EMERY-DREIFUSS MUSCULAR DYSTROPHY USING SKELETAL MUSCLE DIFFERENTIATED HUMAN EMBRYONIC STEM CELLS

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Laminopathies are a wide range of genetic diseases which are caused by mutations in LMNA encoding for Lamin A & C intermediate filament proteins which form part of the nuclear lamina, a dense network of filament and membrane associated proteins in the nuclear envelope. Lamins work in conjunction with other components of the lamina, known together as the LINC complex (LInker of the Nucleoskeleton and Cytoskeleton), to provide the supporting scaffold and physically anchor the nucleus. One such laminopathy is Emery-Dreifuss muscular dystrophy, or EMD, which is clinically characterized by a triad of early contractures in the Achilles tendons, elbows, cervical muscles, progressive muscular atrophy, and cardiac defects. EMD has two modes of inheritance: X-linked and autosomal dominant which are produced by missense mutations in the EMD gene, encoding the nuclear transmembrane emerin protein, or LMNA, respectively. Most studied forms of the disease are caused by missense mutations which have been linked

to mislocalization of the affected proteins and abnormal nuclear morphology. Despite being first described in the early 1900s the pathogenic mechanisms of laminopathic EMD, have only begun to emerge. In addition, there is currently no treatment resulting in progressive muscle weakness and cardiac problems for those afflicted. Through a collaboration with Genea Biocells and San Diego State University's Bridges to Stem Cell Research Internship Program (BSCRIP), we present the development of a human stem cell model for skeletal muscle impairment due to a missense mutation for autosomal dominant EMD. We show a distinctive nuclear morphological phenotype previously described in the literature and a novel reduction in basal ATP levels in resting cells in our differentiated myotube cultures. Using disease-affected pluripotent stem cell lines, a scalable myogenic differentiation protocol, high-content and oxidative stress screening platforms we present a clinically relevant cell-based drug screening model for EMD affected skeletal muscle.

Funding Source: California Institute for Regenerative Medicine Stem Cell Internship Program & Genea Biocells

W-3071

EFFICIENT GENERATION OF HUMAN DISEASE SPECIFIC INDUCED NEURAL STEM CELLS THROUGH OPTIMIZATION OF REPROGRAMMING CONDITION

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Efficient generation of human disease specific induced neural stem cells (iNSC) is essential to find the fundamental causes of nervous system disorders and the new treatments. To establish high efficiency in iNSC generation, it is indispensable to precede optimization of diverse reprogramming conditions. This study aims to find a defined condition which establishes high efficiency in iNSC generation originating in human Niemann-Pick type C dermal fibroblast (hNPCDF), which occurs by the decrease of metabolism function of cellular cholesterol due to mutation of the NPC1 gene. Among added ingredients of culture medium it turned out that the stability of epidermal growth factor, the essential ingredient for iNSC generation, did not decrease in spite of having been exposed to 37oC for 48 hours. On the contrary, the stability of basic fibroblast growth factor reduced even after 24 hours. This could be overcome by adding heparin in the reprogramming medium. Furthermore, after introducing a reprogramming factor to the cell, phenotypic change was possible only in low multiplicity of infection (MOI) but impossible in middle or high MOI. When a reprogramming factor was introduced in hNPCDF in low MOI in the medium added with heparin, it was confirmed that high level of reprogramming efficiency (colony number per initial cell number at day 14) was shown up to about 1.3%. hNPCDF derived iNSC was multipotent and self-renewable. In conclusion, the precise optimization of reprogramming condition made it possible to produce disease-specific iNSC of high efficiency. Disease-specific iNSC will be very useful for disease modeling and drug screening aiming at finding the etiology and treatments of diseases.

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W-3073

GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FROM LYMPHOBLASTOID CELL LINES USING THE SENDAI VIRUS

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Research using patients-derived pluripotent stem cells (hiPSCs) have increased exponentially during the last decade in which they represent a new tool for disease modeling and high-throughput drug screening. Initially, dermal fibroblasts (DFs) isolated from skin biopsies, an invasive method, were used to generate hiPSCs, which may limit the number of samples. To overcome this limitation, hiPSCs were derived from less invasive procedures such as blood and urine samples. An alternative, yet underutilized, bioresource for hiPSCs generation is the immortalized lymphoblastoid cell lines (LCLs), available in worldwide repositories. Until now, hiPSCs were produced from LCLs using episomal (epi) method. We have successfully derived hiPSC from LCLs (LCL-hiPSCs) using the Sendai virus (SeV), a non-integrative method. The advantages of SeV reprogramming compare to epi procedure are higher efficiency and reliability with a low workload and the complete disappearance of viral sequences with passages. LCL-hiPSCs expressed pluripotency markers, maintained the donor identity and could spontaneously be differentiated into the three germ layers in vitro. More importantly, there was no detection of the EBV episomal vectors and the SeV transgenes in the LCL-hiPSCs. Here, we demonstrated the efficient hiPSCs generation form three myotonic dystrophy type 1 (DM1) LCLs and three controls. DM1 LCL-hiPSCs retained the CTG repeats and can be differentiated into neurons (LCL-hiPSC-N) and cardiomyocytes (LCL-hiPSC-CM) in a similar way than DF-hiPSCs. Both differentiated cell

types expressed the CTG repeats and nuclear foci, the hallmark of DM1 pathogenesis. LCL-hiPSC-N and LCL-hiPSC-CM recapitulate most characteristics of native human neurons and cardiomyocytes thus making LCLs an important source for the derivation of hiPSC for drug screening and disease modeling specifically for rare and devastating disorders.

W-3075

RECOVERY OF GENOMIC STABILITY BY ZSCAN10 IN INDUCED PLURIPOTENT STEM CELLS FROM AGED DONORS

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Induced pluripotent stem cells (iPSC), which are generated from a patient's own cells and used to produce transplantable tissues, may particularly benefit older patients who are more likely to suffer from degenerative diseases. However, iPSC generated from aged donors (A-iPSC) exhibit higher genomic instability, defects in apoptosis, and a blunted DNA damage response compared to iPSC generated from younger donors (Y-iPSC). This raises significant safety concerns, as transplantable tissues produced from A-iPSC may be functionally impaired and carry a higher risk of cancer development. When we consider the complex genomic and epigenetic variations that occur during aging, the genomic instability in A-iPSC is likely caused by multiple mechanisms. Here, we describe the discovery of one mechanism that contributes to A-iPSC genomic instability. We demonstrated that A-iPSC exhibit excessive glutathione-mediated reactive oxygen species (ROS) scavenging activity, which blocks the DNA damage response and apoptosis and permits genomic instability. We found that the pluripotency factor ZSCAN10 is poorly expressed in A-iPSC and addition of ZSCAN10 to the four Yamanaka factors (OCT4, SOX2, KLF4, and c-MYC) in iPSC reprogramming normalizes ROS/glutathione homeostasis and the DNA damage response and recovers A-iPSC genomic stability. We confirmed the presence of this mechanism in both mouse and human models, indicating that ZSCAN10 function is evolutionarily conserved. Restoring the genomic stability of A-iPSC will ultimately enhance our ability to produce histocompatible functional tissues from a patient's own cells that are safe for transplantation.

W-3077

QUANTITATIVE PHASE IMAGING AND ANALYSIS OF LIVE INDUCED PLURIPOTENT STEM CELL COLONIES

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Induced pluripotent stem cells (iPSCs) are reprogrammed cells with varying biological potential for use in cell therapy products. Current methods to characterize and identify desired iPSC colonies involve the use of traditional Zernike phase contrast microscopy, which provides qualitative information about cellular features. We demonstrate the use of quantitative phase imaging (QPI) to characterize iPSC colony heterogeneity based on dynamic colony mass and area measurements. Unlike traditional phase contrast microscopy, QPI provides quantitative phase measurements which can be traced back to a reference material. ND2.0 iPSCs were thawed and seeded in six well plates in Life Technologies E8 Flex culture media and expanded for three days. QPI images were taken using a Phasics SID4BIO camera and Zeiss Axiovert 200M microscope. Images were acquired over a large field of view by tiled acquisition at 10x magnification to capture multiple colonies. QPI imaging was performed every hour over the course of three days to track colony proliferation. Image processing and analysis was performed to quantify changes in colony area and mass. Colony doubling time were determined by exponential fit to the mass and area measurements. Polymethyl methacrylate beads were placed in a separate well and immersed in BioUltra mineral oil. Image processing and analysis was performed to determine bead diameter and refractive

index. Colonies (n=14) had a mean mass doubling time of 37.5 ± 10.2 (standard deviation) hours and a mean area doubling time of 61.3 ± 38.0 hours. Doubling times determined from mass and area measurements were statistically different. Bead diameter and refractive index measurements determined from QPI were within reference specifications. iPSC heterogeneity was characterized based on mass and area measurements derived from QPI. While both traditional phase contrast and QPI methods can provide doubling times based on area, mass doubling times can only be determined by QPI and can provide a new method to characterize iPSC heterogeneity. Colonies with high mass proliferation can characterize more pluripotent stem cells with higher rates of self-renewal. Conversely, colonies with low mass proliferation can characterize less pluripotency as senescent or spontaneously differentiating cells.

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W-3079

GENOME MANIPULATION OF INDUCED PLURIPOTENT STEM CELLS USING CRISPR/CAS9

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Clustered regularly interspaced short tandem repeats (CRISPR) and CRISPR associated protein 9 (Cas9) have made genome engineering in mammalian cells possible for laboratories with basic molecular biology capability. Many cell types are relatively easy to manipulate. However, engineering pluripotent stem cells (PSCs) is still insufficient and difficult to achieve. Here, we provide a method to sufficiently obtain genome-edited human PSC lines and minimize the amount of clones to be screened. Many types of genome engineering including knock-in, knock-out, and point mutation can be achieved with this method. Genomic knock-out can be achieved with two CRISPR guide RNA flanking the target region. Genomic knock-in can be achieved by a plasmid donor with 800-1000 bps homologous arms flanking the knock-in sequence. Point mutation can be achieved by a single-stranded oligonucleotide (ssODN) as a donor template. Temporary antibiotic selection greatly increases the successful rate of editing events by eliminating cells without the CRISPR/Cas9 plasmid. We demonstrate that this method is suitable for most laboratories to perform gene-editing in human PSCs and sufficiently obtain modified PSCs clones.

W-3081

ACCUMULATION OF GENETIC ALTERATIONS IN HUMAN PERIPHERAL BLOOD DERIVED MESENCHYMAL STROMAL CELLS DURING IN VITRO CULTURE REVEALED BY WHOLE GENOME SEQUENCING

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Human mesenchymal stromal cells (MSCs) have served as a major cellular resource for cell-based immunomodulatory and regenerative therapies. However, genomic instability may accumulate during ex vivo expansion of MSCs, thereby increasing the potential of malignant transformation. Here, we performed whole-genome sequencing of two peripheral blood-derived MSC lines (MSC1 and MSC2) at various passages (passage 1 [P1] to P9). The majority of single-nucleotide variations (SNVs) occurred in later passages; specifically, 90% and 70% of all SNVs in MSC1 and MSC2 were observed in P9 and P7/P9, respectively. These late-occurring SNVs were enriched with C>A transversions and were overrepresented in intronic regions compared to intergenic regions, suggesting that the mutational forces are not constant across the passages. Clonality analyses also distinguished early-occurring, subclonal SNVs from late-occurring, clonally fixed SNVs. In addition, MSCs were largely devoid of copy number alterations (CNAs) (i.e., 0-2 CNAs per passage), with one exception (MSC2-P3) harboring 29 passage-specific CNAs. Our findings suggest that the SNVs found to be abundant at later passages likely resulted from the accumulation of replication stress, which can be associated with proliferation activity. Thus, the genomic instability associated with proliferation records should be considered for clinical applications of MSCs.

Funding Source: This research was supported by a grant (14172MFDS974) by the Ministry of Food and Drug Safety in 2016 and by a grant (HI15C3076) from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), which is funded by the Ministry of Health & Welfare, Republic of Korea.

W-3083

TOWARD A WHOLE-CELL MODEL OF H1 HUMAN EMBRYONIC STEM CELLS: A GENOME-SCALE METABOLIC MODEL

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Effective use of stem cells for regenerative medicine requires the capability to reliably control stem cell behaviors such as self-renewal and differentiation. Such behaviors result from complex interactions among signaling, gene regulation, metabolic, and other intracellular pathways. Despite extensive research which has elucidated many of the details of these pathways and their interactions, we still do not have a comprehensive, predictive understanding of how these pathways collectively determine stem cell behavior. To gain an integrated understanding of stem cells, we are developing a whole-cell computational model of the H1 hESC line which will represent multiple intracellular pathways including signaling, gene regulation, and metabolism and will be based on a wide range of genomic and biochemical data. We have chosen to focus on H1 hESCs because they are relatively well-studied and karyotypically normal. We plan to develop the model by combining multiple submodels of individual pathways. (1) We have constructed an H1 reference genome by mapping published H1 WGS reads onto the hg19 reference genome. The H1 reference genome will provide the foundation for our model, including the genes, interactions, reactions, and cell composition represented by the model. (2) We have developed a metabolism submodel. (a) We used the Recon 2.2 consensus human metabolic model and proteomic data to construct a core network of likely active metabolic reactions. (b) We used CORDA2 to incorporate the smallest number of additional reactions needed to generate a model that recapitulates the observed production of nucleic acids, amino acids, lipids, and other metabolites required for H1 hESC growth. (3) We plan to create additional submodels of H1 hESC gene regulation, RNA and protein synthesis and degradation, replication, signaling, and cell division and combine the submodels into a single model. (4) We plan to validate the model by testing its ability to reproduce observed H1-hESC phenotypes such as its short G1 phase and rapid growth rate. Ultimately, we plan to use the model to help elucidate the mechanisms which control stem cell self-renewal and pluripotency maintenance. In summary, we believe whole-cell models of hESCs will be powerful tools for understanding and engineering stem cells.

Funding Source: This work is supported by NIH MIRA award R35 GM119771 and NSF INSPIRE award 1649014.

W-3085

MEDICAL STUDENT GUIDE TO PROFICIENCY IN REGENERATIVE MEDICINE AND SURGERY: THE MAYO CLINIC EXPERIENCE

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Discoveries in regenerative medicine have begun to permeate clinical practice yet this dawning era of modern healthcare exposes a knowledge gap in medical education. Herein we describe a successful regenerative sciences education program incorporated into medical school curriculum as a preliminary model for physicians-in-training. In collaboration with the Mayo Clinic Center for Regenerative Medicine, the Mayo Clinic School of Medicine launched its inaugural regenerative medicine and surgery course for first-year medical students in 2014. Course content encompassed specific educational objectives such as understanding fundamental regenerative medicine principles, describing surgical techniques utilized in clinical trials, recognizing the importance of pre-clinical models for testing feasibility, safety and efficacy and ultimately recognizing the steps to bring discovery into the clinic through commercialization and community outreach. Inaugural funding ($100,000) from Regenerative Medicine Minnesota (2016-2017) allowed us to engage over 40 medical students across Minnesota. Five medical students were also funded to attend the 2016 World Stem Cell Summit for poster presentations and networking opportunities. Activities of course alumni include Clinical and Translational Science (CCaTS) Master's Degrees (n=3), Howard Hughes Medical Institute (HHMI) Research Fellowship (n=1), Fulbright Fellowship (n=1) and Ph.D. thesis work (n=5) in regenerative medicine research. Collectively, we have enrolled over 100 medical student trainees. Participation in this course increased medical student awareness of regenerative medicine model of healthcare and contributed to specialty identification, research engagement, and clinical practice. This novel medical school curriculum promotes physicians-in-training to think differently using a restorative approach to healthcare and engineer innovative solutions to address the major unmet needs for patients.

Funding Source: Regenerative Medicine Minnesota

LATE BREAKING POSTER SESSION I-EVEN 19:30 – 20:30

W-3002

MAF1 PROMOTES THE INDUCTION OF EMBRYONIC STEM CELLS INTO MESODERM AND THE DIFFERENTIATION OF ADIPOCYTES.

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Maf1 is a transcriptional repressor that regulates both RNA polymerase (pol) II-and III-dependent genes. While deregulation of Maf1 and RNA pol III-dependent transcription play important roles in oncogenesis, relatively little is known regarding their functions in cellular differentiation or development. Therefore, we examined a potential function for Maf1 in maintaining mouse embryonic stem cell (ESC) properties. While alterations in Maf1 expression did not affect ESC self-renewal, mesoderm induction was impaired. These results indicate that Maf1 is important for driving mesoderm induction. As Maf1 negatively regulates intracellular lipid accumulation, and adipocytes are one of the derivatives of mesoderm, we examined whether alterations in cellular Maf1 could affect adipogenesis. Reduced Maf1 expression in either ESCs or 3T3-L1 preadipocytes resulted in an impairment of adipogenesis while ectopic expression of Maf1 in Maf1-/- MEFs enhanced adipocyte differentiation. Selective repression of RNA pol III-mediated transcription by either chemical inhibition or knockdown of Brf1, an RNA pol III-specific factor, significantly increased pro-adipogenic gene expression and adipogenesis. These results support the idea that repression of RNA pol III-dependent transcription drives adipogenesis. RNAseq analysis revealed that cells with compromised RNA pol III-mediated transcription or altered Maf1 expression produce a significant enrichment of adipogenic gene signatures. Further analysis revealed that RNA pol III-dependent transcription repression reduces the expression of non-coding (nc) H19 RNA and Wnt6. Given that both ncH19 and Wnt6 are established negative regulators of adipogenesis, RNA pol III-mediated repression of these genes likely contribute to its ability to control adipogenesis. These studies establish an unexpected role for Maf1, and the repression of RNA pol III-mediated transcription, in adipocyte differentiation.

W-3004

VASCULAR STEM/PROGENITOR CELL MIGRATION IN RESPONSE TO CHEMOKINES, IN VASCULAR DISEASES - THE EMERGENCE OF A NOVEL CHEMOKINE-LIKE PROTEIN

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Stem/Progenitor cells, such as Sca-1+ cells, are abundant in the vascular adventitia and can differentiate into SMCs and ECs. These cells participate in the formation of vascular lesions including atherosclerosis, injury-induced neointimal hyperplasia, vein graft atherosclerosis and others. The detailed mechanism of progenitor cell migration towards the intima has not been fully understood. We hypothesize that Sdf-1α and Dkk3 play an important role in Sca-1+ vascular progenitor cell migration. Studies have proved that these 2 proteins are released by platelets under inflammatory conditions, thus recruiting cells. Additionally, they are not only highly expressed in SMCs but they can also induce differentiation of stem cells into SMCs. In this work, mouse vascular progenitor cells were isolated from vascular adventitia and sorted for the Sca-1 marker. Using transwell and wound healing assays, we showed that SDF-1α and Dkk3 induce Sca-1+ adventitia-derived progenitor cell (Sca-1+ APCs) migration in vitro. The aortic ring assay was performed to study ex vivo the cell outgrowth induced by SDF-1α and Dkk3, with immunofluorescence characterization of the migrated cells. We observed that more Sca-1+ cells outgrew from the aortic rings upon Sdf-1α and Dkk3 stimulation, particularly in ApoE -/- mice, and a greater number of cells was stained with both Sca-1+ and SMC markers. Analysis of the signaling pathways disclosed that MAPK kinases, PI3K/Akt pathway and Rho-GTPases are potentially involved in the Sca-1+ progenitor cell migration mechanism driven by either SDF-1α or Dkk3. Studies have confirmed that SDF-1α binds to CXCR4 and CXCR7. No receptor has been found yet for Dkk3. We hence sought to identify the receptor to which Dkk3 binds. Co-IP analysis and Affinity binding assay revealed that Dkk3 binds to a chemokine receptor, whose downregulation by SiRNA transfection reduced the Dkk3-mediated Sca-1+ progenitor cell migration and affected the signalling pathways previously described. Dkk3 is a novel chemokine-like protein, with an important role in the migration behavior of the Sca-1+ APCs, sharing with Sdf-1α similar migration mechanisms. The identification of Dkk3's receptor and the dissection of the downstream signalling pathways give rise to insights in the development of novel therapies for vascular diseases.

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W-3006

TISSUE SPECIFIC ENDOTHELIAL CELLS IN DEVELOPMENT

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Vascular endothelial cells (ECs) are the frontiers of organ tissues exposing to the circulating blood. The functions of ECs are not limited to lining the structures of vasculature. ECs also play multiple roles in organogenesis and involve in the regulation of biological processes. Although ECs show some common features, tissue-specific ECs display the morphological distinction and specific gene signatures, which coordinate with organ cells to form unique structures and preform particular functionalities. In lung, pulmonary ECs parallel with epithelial cells to form gas exchange units, which supply the oxygen and discard the waste. In brain, ECs associate with brain cells to develop semipermeable blood-brain barrier, which protects neuronal environment and balances the biochemical regulations. In liver, the connections between ECs are discontinuous allowing the hepatocytes to easily communicate with bloodstream for toxin clearance and nutrition supply. Although previous studies show the different phenotypes and functions in different tissue-specific ECs, it is unknown whether tissue-specific ECs differentiate from the same origin as their coordinated organ cells. In this study, we found that tissue-specific ECs express tissue-specific markers at early developmental stages in lungs, brain and liver. Suppression of ECs with tissue-specific manner decrease ECs population in lungs. Together, our data suggest that ECs and organ cells may differentiate from same origins toward different commitments.

W-3010

STRANGLING NUCLEI: FORMATION OF MICROTUBULES BUNDLES AROUND THE NUCLEUS CORRELATE WITH NUCLEAR DEFORMATION AND CHROMATIN REORGANIZATION IN DIFFERENTIATING HUMAN HEMATOPOIETIC STEM CELLS.

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Chromatin state is highly influenced by nuclear envelope architecture. Modulating nuclear geometry can affect chromatin organization and thus potentially affect gene expression. It has been demonstrated that the actin cytoskeleton is the main actor responsible for nuclear deformation via its ability to transmit forces to the nucleus in adherent cells, but this phenomenon has never been addressed in non-adherent cells. Also, the role of these deformations on stem cell differentiation has never been entirely deciphered. Our study aims to understand the parameters controlling nucleus shape change and what impact it can have on cellular differentiation using a non-adherent stem cell model, the hematopoietic stem cell (HSC). To address this question, nuclear morphology and cytoskeleton organization have been quantitatively analyzed in two different populations of HSCs: one which represents naïve stem cells, identified with the presence of the surface marker CD34 and the absence of the CD38 marker, opposed to early-stage differentiated cells, defined as cells expressing CD34 and CD38. We show that both size and shape of the nucleus are strikingly different in these two populations. Mainly, the nucleus of naïve cells is spherical, while it displays concave surfaces in differentiated cells. These differences correlate with changes in heterochromatin spatial distribution, indicative of chromatin reorganization, which in turn is known to occur during differentiation. Furthermore, cytoskeleton organization was analyzed and the main differences were observed for the microtubules. Interestingly, in contrast to naïve cells, differentiated cells exhibit bundling microtubules tightly apposed to the nuclear envelope. This reorganization suggests strong interaction between microtubules and the nuclear envelope that may be the cause of its deformation. Taken together, these observations suggest that microtubules may monitor nuclear shape changes during early steps of differentiation of HSCs allowing the cell to change its expression program. Modulation of microtubules dynamics and/or interaction with the nucleus could be a key point in controlling HSCs' stemness over time.

W-3012

RNASEQ ANALYSIS OF HEPATOCYTE-LIKE CELLS DIFFERENTIATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS BY DIFFERENT CONDITIONS

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The generation of human hepatocytes with adult characteristics from a renewable source such as human pluripotent stem cells (hPSC) is needed in the context of in vitro disease modeling, drug discovery, toxicology and cell therapy. One of the major problems with existing protocols to generate hepatocytes, whether transcription factor or soluble factor driven, is that they tend to produce hepatocyte-like cells (HLCs) with immature or fetal characteristics, rendering them less than ideal for studying adult liver pathophysiology. Because published methods generally lack a comparison with other protocols, we compared 3 commonly used methods for their ability to produce cells most closely related to their primary human counterparts. In this study, we differentiated induced pluripotent stem cells (iPSCs) into HLCs utilizing a soluble factor based 2-dimensional (2D) monolayer and 3-dimensional (3D) aggregate format in combination with a transcription factor based approach with forced expression of key hepatocyte regulators. Using adult primary hepatocytes as our gold standard, we compared global expression profiles of the differentiated populations using RNAseq. This data suggests that 3D systems supported HLC maturation with increased expression of mature hepatocyte genes including Albumin and numerous liver specific enzymes compared to 2D monolayer or transcription factor overexpression methods. Importantly, the Cytochrome p450 expression levels in the iPSC derived HLCs correlate more closely to mature hepatocytes than many commonly used hepatocarcinoma lines, making iPSC derived HLCs a more attractive alternative for use in liver disease modeling and drug metabolism studies.

W-3014

ANALYSIS OF THE INTERACTING PROTEINS OF GASDERMIN A3, A CAUSATIVE GENE OF SKIN INFLAMMATION AND ALOPECIA

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Skin serves as a critical physical and immune barrier to maintain internal homeostasis of the human body. Murine gasdermin A3 (Gsdma3) was originally identified as a causative gene whose mutations cause skin inflammation and gradual loss of hair follicle stem cells. Gsdma3 belongs to a structurally conserved gasdermin gene family (GSDMA, B, C, D in human) and recent X-ray crystallography analysis on GSDMA3 revealed a two-domain structure connected by a flexible linker. Emerging evidence from our lab and others suggested that the N-terminal domain of gasdermin family contains the cell-death promoting activity, which is auto-inhibited by its C-terminal domain. The cell death mediated by N-terminal Gsdma3 (N-Gsdma3) has been ascribed to its capability to oligomerize and forming membrane pores (pyroptosis), to induce autophagy, or to induce mitochondrial dysfunction. We have previously demonstrated that N-Gsdma3 associates with Hsp90/Hsp70/Hop complex and is delivered to the mitochondria via docking onto Tom70 importer, subsequently leading to mitochondrial dysfunction. Herein, we further explored the role of the N-terminus sequence of N-Gsdma3 on its oligomerization, mitochondrial localization, and cell death promotion. A construct harboring an internal deletion close to the N-terminus of N-Gsdma3 was made. We found that the internal deletion greatly reduced its ability to induce self-oligomerization and cell necrosis. However, the internal deletion construct still induced a reduction in mitochondrial membrane potential. Double immunostaining of the tagged-construct and mitochondrial Tom20 showed that it is still co-localized with the fragmented mitochondria. Furthermore, we used immunoprecipitation and LC-mass spectrometry analysis to identify the interacting proteins of the internal deletion mutant. We identified Hsp90, Hsp70 Hop, TRAP1, and ATAD3A as potential N-Gsdma3-interacting proteins. Co-immunoprecipitation and double immunostaining analyses confirmed the interactions and co-localization with the mitochondria, respectively. Our data suggest that N-Gsdma3 possibly targets critical components in mitochondria to induced mitochondrial dysfunction, and that the degree of self-oligomerization may regulate its cytotoxicity.

Funding Source: MOST 103-2320-B-400-011-MY3, NHRI 05A1-CSPP12-014

W-3016

COORDINATED MOTION OF THE STEM CELL NICHE IS CRITICAL TO RECOVERY AFTER DAMAGE DUE TO IN VIVO FEMTOSECOND LASER ABLATION

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The intestinal epithelium harbors many integral functions, including serving as a barrier to luminal pathogens. Maintaining integrity in response to local damage requires a rapid defensive mechanism. By using in vivo multiphoton microscopy which allows us to monitor real-time events in mice with GFP-labeled Lgr5-expressing crypt base cells, we recently observed a new type of cellular behavior consisting of two types of coordinated motion in response to injuries in the small intestine crypt. To induce local damage, we used femtosecond laser ablation, which can target an individual cell without damaging neighboring cells. Laser ablation resulted in rapid death of the targeted cells, followed by adjacent cells moving towards the target site to "push" cellular debris out of the plane of the crypt base towards the lumen. The crypt lumen then expanded and contracted as the debris moved up the lumen. In young (2 to 8 months) animals, the average speed of debris motion was 13.18 um/hour. The lumen of the crypt expanded its circumference to 145 +/- 40 % of baseline at one hour after ablation as cellular debris moved toward the lumen and returned to baseline within 24 hours. To test whether these motions are active processes, we topically applied Y27632, a ROCK inhibitor that interferes with cellular motility. Y27632 reduced the speed of debris motion to 8.8 um/hour and reduced the increase in crypt circumference to 127 ± 31% of baseline. Interestingly, our preliminary data show similar delays in debris motion (5.55 um/hour) and attenuated lumen dilation in aged mice (18 to 24 months old), with crypt circumference only reaching 115 ± 6 % of baseline. These data suggest that in response to local damage, crypt cells engage in a coordinated, peristaltic motion to actively push cellular debris from the plane of the crypt base toward the lumen. Thus, we have identified a novel mechanism, which may be dysregulated with aging, by which intestinal crypts can respond to a local perturbation in a coordinated fashion to restore homeostasis.

Funding Source: The project described was supported by the Empire State Stem Cell Fund through New York State Department of Health Contract # C30293GG

W-3018

MECHANICAL PHENOTYPING OF PRIMARY AND ESC-DERIVED ROD PHOTORECEPTORS

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Transplantation of photoreceptors is a promising approach aiming to replace or support degenerating photoreceptors as demonstrated in preclinical mouse models. Improved transplantation success and depletion of contaminating cells was achieved in these studies by donor photoreceptor enrichment prior injection using flow cytometry or magnetic activated cell sorting. However, such purification methods require genetic modifications of donor cells or the use of cell surface binding antibodies. Mechanical properties were proposed as an alternative sorting approach circumventing the modification of cells. Here, we characterize the mechanical properties of rod photoreceptors isolated at different developmental stages either from the mouse retina or embryonic stem cell-derived organoids in a contactless, high-throughput manner using real-time deformability cytometry. We show that, independent of their source, rods become smaller, softer and deform less as development proceeds and that their mechanical properties are sufficient to identify rods from other retinal cells within an unlabeled heterogeneous cell population. Hence, this study defines the key rheological parameters that might allow label-free sorting of rod photoreceptors in future therapeutic transplantation approaches.

Funding Source: Deutsche Forschungsgemeinschaft (DGF) FZT 111, Center for Regenerative Therapies Dresden, Cluster of Excellence (M.A.), DFG Grant AD375/3-1(M.A.), the Bundesministerium für Bildung und Forschung (ZIK HIKE grant to O.O.)

W-3020

RAPID DIFFERENTIATION OF ASTROCYTES FROM HUMAN ESCS AND IDENTIFICATION OF THE NOVEL GENES RELATED WITH ASTROCYTE DIFFERENTIATION.

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Astrocytes are the most abundant cells of the human brain and have the role of biochemical support of endothelial cell, supply of nutrients to the nervous tissue and maintenance of extracellular ion balance etc. In development of nervous tissue, the differentiation of astrocytes is later than neurons. In vitro, it takes more time and more techniques to obtain mature and pure astrocytes. In this study, we developed the previous protocol to gain mature and pure astrocytes from human embryonic stem cells. To keep the quality of the differentiated astrocytes and to decrease the variations of the cell properties, we tried to get neural progenitor cells (NPCs) and expand the cell number. By FACS analysis, we found that the percentage of astrocytes among the cells differentiated from NPCs was over 90%. And then we checked the cell property with immunocytochemistry and western blot using antibodies for astrocyte specific marker proteins. Also the functional property of the astrocyte, IL-6 release in inflammatory response and neuronal supporting ability, was tested using ELISA assay and co-culture experiment with neurons. Fallowing our protocol, we can obtain the mature and pure astrocytes within 4 weeks from NPCs. Next, we identify the influential genes related with astrocyte differentiation using mRNA sequencing. By the validation of RT-PCR and western blot assay, we eventually sort out four genes relevant to transcriptional factor. We have the plan to investigate the roles of our target genes in astrocyte differentiation.

W-3022

HISTONE DEACETYLASE INHIBITION-MEDIATED NEUROGENIC DIFFERENTIATION VIA WNT SIGNALING PATHWAY IN HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS

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Histone deacetylase (HDAC) inhibitor has potential effects on cell homeostasis, cell cycle progression, and terminal differentiation. However, the roles and mechanisms of HDAC inhibitors on neurogenic differentiation with a Wnt signaling pathway have not yet been completely elucidated in stem cells. We hypothesized that the HDAC inhibitors regulate downstream Wnt signaling and control stem cell maintenance and neurogenic differentiation. We examined the effect and mechanism of HDAC inhibitors, such as MS-275, sodium butyrate (NaB), trichostatin A (TSA), or valproic acid (VPA), on neurogenic differentiation of human adipose tissue-derived mesenchymal stem cells (hADSCs) using RT-PCR, western blot, and immunocytochemistry. Following neurogenic induction with supplementary factors, hADSCs were differentiated into neuronal cells in vitro. The increase in the number of neurites and neural lineage specific markers was notable when MS-275, NaB, TSA, or VPA incorporated in the medium. The expression of neurofilament-L (NFL) and neurofilament-M (NFM) were highly increased in HDACi treatment compared control medium by RT-PCR. The microtubule-associated protein 2 (MAP2) level was increased after MS-275 or VPA treatment. The expression of Wnt1, Wnt2, and LRP5/6, which are canonical Wnt and Wnt ligands, in hADSCs treated with VPA was significantly greater compared to that of control medium. However, the expression of Wnt5 and Fzd4 were increased with MS-275, NaB or TSA in the medium. There were no changes in the expression of β-catenin and GSK-3β. Interestingly, Wnt3 expression was highly increased in MSCs with VPA and Wnt5a was expressed with MS-275, NaB, or TSA treatment by real time RT-PCR. Wnt5a level was upregulated after neurogenic induction with MS-275, NaB, or TSA treatment by western blot assay. Furthermore, we found that the JNK expression was increased after NaB or TSA treatment, whereas ERK level was decreased. Treatment of MS-275 and VPA upregulated GSK-3β and β-catenin. In conclusion, these findings indicated that HDACi could induce neurogenic differentiation of MSCs by activating canonical Wnt or non-canonical Wnt signaling pathway.

W-3024

A HUMANIZED IN VIVO MODEL OF ZIKA-INDUCED MICROCEPHALY USING HUMAN INDUCED NEURAL STEM CELLS

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Zika virus (ZIKV) is a mosquito-transmitted flavivirus with a causative link to microcephaly, a condition resulting in small cranial size and multiple brain abnormalities. Despite recent progress, there is a current lack of physiologically relevant in vivo models that permit the study of systemic virus on human neurons

in a developing organism. Furthermore, no therapeutic to date has been reported to reduce ZIKV-induced microcephaly. We tested the effects of ZIKV on human induced neural stem cells (hiNSCs) in vitro and found that infected hiNSCs secrete ZIKV, inflammatory cytokines and growth factors; display altered differentiation; and become highly apoptotic. We intracranially injected hiNSCs into developing chick embryos, subjected them to systemic ZIKV infection and found that humanized ZIKV-infected embryos developed severe microcephaly. Lastly, we utilized this humanized model for subsequent drug studies, and identified an FDA-approved treatment that can partially rescue ZIKV-induced microcephaly and attenuate infection of hiNSCs in vivo.

W-3026

DISEASE MODELING WITH HUMAN PLURIPOTENT STEM CELL-DERIVED MOTOR NEURONS REVEALS A CONNECTION BETWEEN SEVERAL ALS PATHOGENIC MECHANISMS

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ALS is a devastating disease characterized by the loss of motor neurons. The identification of genetic triggers has ushered in an era of molecular discovery for ALS, and emerging themes include dysfunction in RNA metabolism and protein homeostasis, the induction of ER stress, altered glial environment, and disruptions to the cytoskeleton. Additionally, a common pathological hallmark in most ALS cases is the presence of cytoplasmic inclusions of TDP-43. An unresolved mystery is: why do mutations in the over 20 genes associated with diverse cellular pathways converge on the same pathological hallmark? TDP-43 is a primarily nuclear DNA/RNA binding protein with functional roles in transcriptional regulation, splicing, pre-miRNA processing, stress granule formation, and mRNA transport and stability. Whether degeneration linked to TDP-43 is the result of loss-of-function, gain-of-function, or both mechanisms remains unclear. The inaccessibility of human motor neurons combined with our inability to expand them in culture has impeded the types of studies needed to address this question. Because stem cells can be directed to differentiate into motor neurons, we reasoned that interrogation of human genes differentially expressed after TDP-43 dysregulation in the cells affected in ALS could provide novel insights. Accordingly, we combined stem cell differentiation approaches with RNA interference and RNA sequencing technologies to investigate global changes in gene expression levels in motor neurons after dysregulation of TDP-43. Notably, we discovered that TDP-43 levels are required to sustain both the mRNA and protein levels of the neuronal-growth associated factor STMN2, a critical component of the cytoskeleton. We also demonstrated that proteasome inhibition, another ALS-relevant stimuli, can trigger TDP-43 mislocalization and decreased levels of STMN2. Thus, our results provide a link between proteostasis, RNA metabolism, and the cytoskeleton leading us to propose a molecular mechanism whereby disruptions to the proteasome can trigger a loss of TDP-43 function in motor neurons, which in turn depletes STMN2 levels to contribute to the development and progression of ALS. In conclusion, discoveries from human stem cell-based models can be of relevance to the underlying pathomechanisms of ALS.

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W-3028

AUTOPHAGY DEFECTS IN A HUMAN NEURAL MODEL OF SPINAL AND BULBAR MUSCULAR ATROPHY

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Spinal and bulbar muscular atrophy (SBMA) is a neuromuscular degenerative disease caused by a CAG trinucleotide repeat expansion in the androgen receptor (AR) gene. When translated, the resultant expanded polyglutamine tract causes AR aggregation and toxicity when in the presence of androgen hormone. While these aggregates may be degraded by macroautophagy, recent studies have purported to show deficits in autophagic activity in SBMA models, although the precise nature of these defects remain elusive. In order to investigate further, several new isogenic pairs of genetically altered human iPSCs with varying polyglutamine lengths have been created and differentiated into motor neuron-like cells. This model immediately permits interrogation of disease-relevant molecular pathways, and ongoing research has identified dysregulation of upstream macroautophagy induction and regulatory processes.

W-3030

STUDIES OF THE INITIATION OF SMALL CELL LUNG CANCER USING CELL DERIVED BY DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

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To ask why cancers arising in different tissues have characteristic genotypes, we are developing methods to study the initiation of human cancers by genetically modifying cells derived from human embryonic stem cells (hESCs) at discrete stages of differentiation. We have focused initially on small cell lung cancer (SCLC), the most aggressive type of human lung cancer, characterized by a poor prognosis and the rapid development of resistance to treatment. The genetic changes important for the initiation and progression of SCLC are poorly understood, in part because biopsies of early stage disease are difficult to obtain and because the many mutations found in mature tumors complicate analyses. To prepare cell cultures with high proportions of pulmonary neuroendocrine cells (PNECs), the putative precursors of SCLC, we have extended published methods for inducing hESCs chemically to form parts of the pulmonary lineage (Huang, et.al, Nat Biotechnol, 2014. Huang, et.al, Nat Protoc, 2015). By inhibiting the gamma-secretase-mediated cleavage of NOTCH receptors to interfere with NOTCH signaling and by inducing inhibitory RNA to reduce expression of the RB1 gene (one of the two tumor suppressor genes most commonly inactivated in SCLC), we have produced hESC-derived cultures in which 40 percent of the cells are PNECs, as defined by the presence of the neuroendocrine marker, CGRP. Single cell RNA profiling shows that the hESC-derived cells express several other genes characteristic of mature human PNECs, including PGP9.5, NCAM1, PC1, the transcriptional factor gene ASCL1, and the lung lineage-specific gene NKX2.1. We are now perturbing genes, in addition to RB1, that are known to be mutated in SCLC to determine the combinations of changes that can produce tumors resembling SCLC when the manipulated cells are implanted in immunodeficient mice.

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W-3032

GERM STEM CELL DEVELOPMENT, CHROMATIN REGULATION AND GENE TRANSCRIPTION BY THE INNER NUCLEAR MEMBRANE PROTEIN SPEG

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The Drosophila testis is a tubular structure that is constantly replenished by renewal of stem cells. All stages of sperm development can be observed in the testes, with more mature stages located furthest from the apical hub, where germ stem cells (GSC) and somatic cyst stem cells (CSC) reside. We have previously identified sperm and egg-less (speg, CG9723) as an inner nuclear membrane protein crucial for fertility. In speg testes, the hub is present, CSC can differentiate into early and late cyst cells (CC) but they are distributed further away from the hub than in control flies. Germ cells (GC) are reduced in number and not tightly packed in cysts of speg larval testes and adult testes often lack GC. At the cellular level, the nuclear envelope has a distorted appearance in speg testes. Since the nuclear envelope and lamina have been implicated in chromatin organization and transcription, here, we explore if speg plays a role in chromatin regulation and gene transcription. Interestingly, heterochromatin protein 1 (HP1) is decreased in both speg mutant testes and wing discs. H3K4me3, H3K9me3, H3K27me3 and core histones are also decreased in speg mutant wing discs. To determine if Speg interacts with specific chromatin loci, we performed DamID in Kc cells. We found that Speg associates with mostly repressive (black) chromatin. The target genes of Speg form gene cluster with the chance higher than random permutation. Intriguingly, the target genes are highly enriched in testes. We further performed ATAC-seq and RNA-seq in wing discs and found that chromatin accessibility is affected and some of the affected regions correlate with gene expression changes in speg mutant. Overall, we propose that modulating gene expression may be one of the possible functions of speg in germ cell development.

W-3034

FUNCTIONAL HIGH ASPECT RATIO POROUS SILICA NANONEEDLES CAN DIRECT CELLULAR FATE

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The cellular niche is a complex ensemble of soluble factors, extracellular matrix and specific mechanical stimuli regulating fundamental cellular functions. The biophysical cues arising from the extracellular space propagate to the nucleus via the direct physical linkage that exists between the cytoskeleton and the nuclear envelope, modifying transcriptional patterns. In this study, we investigated the role of biophysical cues in the reprogramming of somatic cells. To address this aim, mouse embryonic fibroblasts (MEFs) were cultured on a high aspect ratio nanomaterial, the nanoneedles arrays (nN). nN have been shown to produce sharp invagination of the cell membrane and profound changes in the cytoskeleton leading to deformation of the nuclear structure. RNAseq performed on MEFs grew on nN or tissue culture plastic revealed a significant increase in the gene sets associated with chromosomes organisations, nuclear division and cell cycle in cells cultured on the nN. These results were mirrored by the Label-free Mass Spectrometry proteomic analysis. Further investigation by Spectral Illumination Microscopy showed that cells on the nN displayed a noticeable reduction in the nuclear volume, number and size of the heterochromatin foci, indicating functional re-arrangement of the chromatin into a more decondensed state. Flow cytometry analysis of chromatin compaction and FRAP of Histone-1 confirmed that cells grown on nN presented a more relaxed conformation. Interestingly, analysis of the differentially expressed genes against the Epigenetic Factor databases revealed alterations in the histone methylation complex, in particular, the Polycomb Repressive Complex 1. RT-PCR analysis confirmed these results and showed a pattern of changes in the histone-modifying enzymes which was reflected by a significant reduction in the level of repressive H3K27me3 marks and an increase of the activating H3K4me3, as shown by Western Blot. Moreover, the level of DNA 5-mC was also significantly reduced. The changes observed in the nuclear shape, DNA organisation and RNA/protein expression indicates the induction of a responsive and open state in the cells grown on nN. Next, we sought to evaluate if these changes can promote a favourable conformation for reprogramming, leading to higher iPSC generation efficiency.

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W-3036

FOXG1 OVEREXPRESSION AND EPIGENETIC LANDSCAPE IN A HUMAN IPSC-DERIVED FOREBRAIN ORGANOID MODEL OF SEVERE, MACROCEPHALIC AUTISM SPECTRUM DISORDER

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Autism spectrum disorder (ASD) is a highly heritable developmental condition associated with early embryonic cortical overgrowth, macrocephaly, and GABAergic neuronal and synaptic imbalance. Altered expression of FOXG1, a transcription factor involved in early forebrain patterning, has been implicated in syndromic ASD. Our previous publication showed that hiPSC-derived forebrain organoids from macrocephalic ASD probands show coincident FOXG1 overexpression, cell cycle acceleration, and GABAergic predominance compared with organoids derived from these ASD probands' unaffected fathers; FOXG1 shRNA knockdown in ASD-derived organoids by RNAi normalized many ASD-associated gene network and neurophenotypic changes. However, to what extent FOXG1 upregulation specifically contributes to ASD, and the details of the FOXG1 regulatory network in health and disease, remain unclear. Additionally, the epigenetic landscape of ASD is not well understood, particularly with regard to FOXG1. Our previous ASD-derived organoids showed increased FOXG1 expression but without mutations in the FOXG1 sequence or upstream promoter regions, suggesting that epigenetic or distal regulatory changes might contribute to these patients' ASD phenotype. First, this project compares ASD-derived organoids with doxycycline-inducibly FOXG1-transactivating organoids derived from these probands' unaffected fathers, as well as with age-matched unrelated controls. RNA-sequencing (RNA-seq), immunocytochemistry, and cell cycle length analysis will be used to explore the extent to which FOXG1 overexpression in these non-ASD organoids recapitulates the ASD organoid phenotype. Second, this project includes ChIP-seq experiments on organoids derived from ASD patients and their unaffected fathers at various developmental time points, and has produced further insight into the epigenetic landscape and pathology of the developing brain in ASD. FOXG1 over-expression experiments have

already yielded unexpected and interesting results suggestive of a nuanced FOXG1 regulatory environment in pluripotency and neuronal differentiation. This set of experiments should better illustrate the contribution of FOXG1 and chromatin state to Autism Spectrum Disorders.

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W-3038

PREMATURE INHIBITION OF WNT SIGNALING BY HIGH DOSES OF DKK-1 IN A SCAFFOLD SEEDED WITH AUTOLOGOUS BM-MSC DIFFERENTIATED TO CHONDROCYTES: POSSIBLE TARGET FOR CLINICAL APPLICATION

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The development of improved methods for treatment of chondral defects using autologous cells in combination with biomaterials leads to a new generation of implantable devices. In order to achieve a biological cartilage repair or regeneration, clinicians and basic researchers have been exploring different alternatives being tissue engineering with different cell types one of the most promising. Chondrocytes are subject to hypertrophic changes, reflected by increased cell volume, increased expression of type X collagen and alkaline phosphatase in the extracellular matrix, thus the use of mesenchymal stem cells (MSCs) differentiated to chondrocytes in vitro may be an alternative to analyze the pathogenesis of cartilage-associated diseases. Chondrogenic differentiation process is regulated by the WNT/β-catenin pathway, which in late stages of differentiation has higher activity, triggering hypertrophic changes. A direct inhibitor of the WNT pathway is Dickkopft-1 (DKK-1) interacting with WNTs receptor ligands. Our purpose was to stablish if different doses of DKK-1 are able to downregulate WNT-related hypertrophic changes in MSC-seeded scaffolds. Scaffolds were optimized in regards to the cell seeding densities and attachment times and different concentrations of DKK-1 were evaluated. DKK-1 was added from day 3 and scaffolds were harvested after 1, 7, 14 y 21 days of induction. Formalin fixed-paraffin embedded scaffolds were stained with hematoxylin/eosin (HE) and toluidine blue (TB) to evaluate cell morphology, cell area and glycosaminoglycan (GAG) synthesis, respectively. Our results revealed that cell seeding occurred between 15±2% - 20±5% in the scaffolds. In the absence of DKK-1, rounded cells with a perinuclear clear halo, features of hypertrophy was observed. In presence of low DKK-1, fewer changes occur than with high DKK-1, which was associated with chondrocyte morphology (decrease in cell area) and increased proteoglycan deposition by analyzing Hematoxilin-Eosin and Toluidine-Blue staining, respectively. In summary, our results show that the premature use of high doses of DKK-1 in autologous BM-MSC-seeded scaffolds may be a possible treatment for more stable and better chondrogenic phenotype able to regenerate cartilage lesions.

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W-3040

CHARACTERIZATION OF TISSUE- AND AGE-SPECIFIC ECM-BASED GELS FOR 3D CELL MICROENVIRONMENT

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The extracellular matrix (ECM) consists of structural and functional molecules secreted by the resident cells. The 3-dimensional organization and composition of an ECM is distinctive for each tissue type. ECM plays a critical role in mediating normal cellular behavior. Solubilized gel-forming ECM could potentially be used to develop products such as 3D culture substrates, injectable materials, and cell delivery vehicles. Currently available commercial products for recreating the 3D environment use xenobiotic or synthetic substrates that do not adequately recapitulate the native niche for human cells. Our goal is to design a 100% allograft custom-fabricated human ECM-derived 3D microenvironment that mimics the biological properties of native ECM. Toward this goal, we evaluated the biochemical and functional characteristics of ECM isolated from human placenta, adipose, and cadaveric muscle tissues. In addition, we compared the properties of muscle-derived ECM isolated from young versus old donors. We found that total protein concentration was comparable in all ECM samples. Detection of soluble collagen was highest in muscle-derived ECM. Placental ECM contained considerably higher amounts of hyaluronic acid compared to ECM from other tissues. Proteomic analysis showed the presence of proteins involved in matrix remodeling, angiogenesis, cell growth and differentiation. ECM protein variability largely depended on tissue type and donor age. Thus, ECM from young muscle contained fewer growth factors than old muscle ECM. Live/Dead assays confirmed a lack of cytotoxicity regardless of the type of tissue or donor age. MTT assays revealed comparable levels of cell proliferation upon exposure to all ECM types in both 2D and 3D settings. Regardless of tissue origin, ECM-based 3D gels affected cell morphology by reducing cell spreading. Differentiation of adipose-

derived stem cells (ADSCs) subjected to ECM sourced from various tissues showed no effect on adipogenic ADSC differentiation. Contrastingly, we found that the presence of young muscle ECM augmented chondrogenic ADSC differentiation, while old muscle ECM favored osteogenic ADSC differentiation. In summary, tissue- and age-related properties of ECM should be considered in developing 3D culture models for basic and translational research.

W-3042

EXPECTATIONS AND CONCERNS REGARDING THE CREATION AND USE OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED GAMETES: A SURVEY OF THE GENERAL POPULATION IN JAPAN

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Research for developing human induced pluripotent stem cell (hiPSC)-derived gametes is ongoing in several laboratories worldwide. While the gametes are expected to have significant applications in research and clinical settings, there are also various concerns with respect to their use. In 2008 the Hinxton Group, an international consortium on stem cells, ethics and law, discussed the scientific situation, ethical issues, and status of regulations in relation to gametes. Recently, in 2016 the British Nuffield Council on Bioethics investigated the ethical issues associated with the creation and use of gametes. Currently, the Cabinet office's Expert Panel on Bioethics in Japan is also deliberating on the ethical issues and regulations associated with this field of research. According to the current regulations, hiPSCs may be used to generate gametes, but these gametes should not be used to create embryos. The panel pointed out the necessity to ascertain the general public's attitude toward gametes. However, the public's perceptions about the creation and use of gametes have thus far not been clarified. Therefore, the aim of this study is to determine the expectations and concerns of the Japanese public with respect to the creation and use of hiPSC-derived gametes. In this study, data will be collected from 2,832 individuals registered with a research company by the end of April 2017 using an online system. Thirteen options are set for expectations, for example, "elucidate human development and genetic mechanisms," "develop therapies or drugs for infertility attributable to sperm or eggs," and "enable anyone who desires a child to have a genetically-related child." Seventeen options are set for concerns, for example, "it is not natural," "the children born through this process will be concerned about their origin and identity," and "it will also fortify the tendency to use embryos as tools." It is expected that this survey will reveal the attitude of the general population in Japan toward expectations and concerns related with the creation and use of hiPSC-derived gametes. These findings will be helpful to better understand the perceptions of the public toward hiPSC-derived gametes and to frame related guidelines based on these findings.

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W-3044

THE PUF BINDING LANDSCAPE IN MALE AND FEMALE GERMLINES

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PUF (Pumilio and FBF) RNA-binding proteins control stem cell self-renewal in diverse species, including mammalian, arthropod and nematode, in addition to other biological functions. The Caenorhabditis elegans PUF protein FBF (fem-3 Binding Factor) serves as a paradigm for metazoan PUFs. FBF is essential for the maintenance of germline stem cells, but also regulates the hermaphrodite sperm/oocyte cell fate switch and is critical for the process of spermatogenesis. We have attempted to "disentangle" the different roles of FBF by comparing its target RNA network in spermatogenic and oogenic germlines. To this end, we used FBF iCLIP (individual-nucleotide resolution UV crosslinking and immunoprecipitation) to determine its binding profile in an adult hermaphrodite germline that is sexually transformed from female to male at the elevated temperature of 25°. As a comparison, we analyzed FBF iCLIP data from oogenic germlines at the same temperature. Using a modified peak-calling algorithm, we identified FBF binding sites in oogenic animals at 20°, oogenic animals at 25°, and spermatogenic animals at 25°. Oogenic FBF targets were similar at 20° and 25°. By contrast, FBF mRNA targets in spermatogenetic animals had a distinct profile, revealing sperm-specific targets that may be critical for the FBF role in spermatogenesis. Roughly 80% of FBF targets were independent of germline gender. In particular, a distinct group of 22 mRNAs were bound at very high frequency (roughly 5% of all FBF-RNA complexes) in a gender- and temperature-independent manner. These 22 mRNAs are enriched for RNA-binding proteins, P granule components, and regulators of gamete generation. All 22 are bound at the known FBF-binding motif. 8/22 are also targets of the human PUF protein PUM2. We suggest this group may represent a major conserved pathway for PUF proteins to maintain stem cells.

W-3046

A FORWARD GENETIC APPROACH TO IDENTIFY NEW MODULATORS OF HEDGEHOG SIGNALING IN MOUSE HAPLOID EMBRYONIC STEM CELLS

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Development in multicellular animals depends on well-orchestrated signal transduction cascades. The Hedgehog (HH) signaling pathway plays a key role in the regulation of embryonic development by governing cell differentiation, proliferation and tissue patterning. HH signaling is conserved in evolution but also displays clade-specific differences. In humans, deregulation of this pathway results in a number of physiological disorders and contributes to the development of aggressive and metastatic cancers. Whereas the general structure of HH signaling has been described, certain key aspects of signal generation and transmission remain to be established. To advance the understanding of HH signaling in mammals, we aim to identify new mediators and effectors of HH pathway performing an innovative forward genetic screen approach in mouse haploid embryonic stem cells (ESCs). Random integration of gene trap vectors in haploid ESCs offers the feasibility to perform genetic screening as in haploid yeasts, but with the great advantage of investigating mammalian pathways. To analyse the effects of insertional mutagenesis on HH pathway activity, we adopted different strategies: analysis of the transcriptional responsiveness of the GLI proteins (final outcome of HH signaling cascade) and haploid ES cells susceptibility to the cytotoxic effects of HH agonistic drugs. It is expected that a genetic approach can unveil factors in the HH signaling cascade able to mechanistically explain inhibition of Smoothened by the HH receptor Patched and downstream events that mediate transcriptional activation of target genes. Discovery of new modulators and effectors of the HH signaling could also have implications for understanding hyper-activation of the HH pathway in human cancers.

W-3048

NEGATIVE REGULATION OF ENDOPEPTIDASE ACTIVITY SPECIFICALLY DRIVE CELL FATE DETERMINATION TOWARDS CELLULAR SENESCENCE

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Cellular senescence is a fundamental status of cells that undergo irregularity such as permanent cell cycle arrest and secreting inflammatory factors after confronting cellular stresses including genotoxic exposure, oncogenic mutation and oxidative stress. Recently it has been illuminated that dysfunctional senescent cells accumulated during the aging process would act as detrimental factors contributing to geriatric diseases via impairing tissue regeneration and triggering the inflammatory response. Therefore, a better mechanistic understanding of cellular senescence towards potential development of anti-senescence therapeutics and/or regenerative medicine could help reduce occurrence of severe geriatric diseases during aging process and ultimately contribute to extend healthy lifespan. Although cellular senescence has been recognized as one of the major fundamental cell fates, the unique signaling pathway underlying this cell fate remain less understood as p53/p21 and Rb pathways also play important roles in other non-dividing cell fates such as quiescence and apoptosis. Our research is aimed to identify crucial “drivers” that may be specifically involved in senescent process. Taking advantage of our recent discovery of epidermal growth factor (EGF) as a natural senescence-suppressor for normal human primary cells grown in culture besides its traditional role as a mitogen to stimulate cell proliferation, we employed an EGFR inhibitor to induce non-dividing cell fates (quiescence and senescence) in normal human bronchial epithelial (NHBE) cells as a model system and compared the gene expression profiles among control, quiescent and senescence-positive population. Subsequently, we have focused our attention on an interesting but less appreciated pathway involving negative regulation of endopeptidases. Specifically, a natural serine protease inhibitor SERPINB4 is upregulated exclusively in senescence-positive cell population and ectopic expression of SERPINB4 in both NHBE and human lung diploid fibroblast cells robustly induced a senescent phenotype, and its expression is also required for senescent programming since cells with blockage of SERPINB4 up-regulation were instead rendered into programmed cell death when challenging with cellular stresses.

W-3050

REDUCED DIPLOIDIZATION AND SURVIVAL IMPROVEMENT OF SEMI-CLONED MICE PRODUCED FROM ANDROGENETIC HAPLOID EMBRYONIC STEM CELLS (AG-HAESCS)

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Androgenetic haploid embryonic stem cells (AG-haESCs) hold great promises in gene function exploration and generation of gene editing semi-cloned (SC) mice. However, high incidence of diploidization and low efficiency of SC mice production from the AG-haESCs are the major obstacles for widely use of these cells. Although the knockout of differentially methylated regions (DMRs) of imprinted genes, H19 and Gtl2, can greatly improve the efficiency of SC mice production, over 40% of the SC mice can not survive to adulthood. Here, we found that the overall DNA methylation and hydroxymethylation in AG-haESCs are extremely low and the downregulation of both de novo methyltransferase Dnmt3b and methylation maintenance enzyme Dnmt1 was discovered responsible for this DNA hypomethylation. Meanwhile, the decreased expression of Tet2 was determined as the major cause for DNA Hypohydroxymethylation in the AG-haESCs. We further discovered that DNA hypomethylation affects both repeat sequences and DMRs of imprinted genes. The overexpression of Dnmt3b1,Dnmt1s and Dnmt3a in AG-haESCs could partially restore the DNA methylation level and most importantly, the high incidence of diploidization can be greatly rescued by Dnmt3b overexpression and subsequently all the SC mice produced could survive to adulthood. Overall, our study discovered that the correction of DNA methylation can greatly reduce the incidence of diploidization and further improve the survival of semi-cloned mice produced from AG-haESCs.

Funding Source: This project is supported by the National Natural Science Foundation of China(31401247, 31401266 and 81630035), the Ministry of Science and Technology of China (grants 2016YFA0100400, 2015CB964503 and 2015CB964800).

W-3052

DEMETHYLATION OF TYPE 1 DIABETIC PATIENT ENHANCES THE DIFFERENTIATION OF INSULIN PRODUCING CELLS

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Type I diabetes is an autoimmune disorder which targets pancreatic β-cells in islets. The pancreatic islets transplantation is the most effective for treatment of T1D patients. However, the shortage of cadaveric islets limits this approach. Recently, induced pluripotent stem cell derivatives are emerging as great sources for cell therapy. Therefore insulin producing cells (IPCs) derived from iPS cells could thus significantly improve patient treatment. Initially, we differentiated T1D iPS cells into IPCs using 3D platform. However, the yield was very poor, and PDX-1 gene expression of T1D-IPCs was significantly lower than in IPCs from non-diabetic iPS cells. Thus, we hypothesized that epigenetic barriers were prevalent in T1D iPS cells, limiting their differentiation into IPCs. To address this problem, we utilized a small molecule to demethylate the DNA methyltransferase (Dnmt). After the differentiation procedure, we found that the cells were demethylated significantly differentiated better than untreated cells. When transplanted in immunodeficient mice that had developed streptozotocin-induced diabetes, there was a dramatic decrease of hyperglycemia, allowing the mice to be normoglycemic within 28 days. Therefore, our data demonstrate that T1D iPS cells can be successfully differentiated into functional IPCs. Our study suggests that 3D culture platform and epigenetic modification could be an efficient strategy for generation of clinically useful IPCs from T1D patient's iPS cells. Additionally, these cells can be used for drug screening, thereby accelerating drug discovery.

W-3054

THE ROLE OF METABOLITES IN DIRECTING STEM CELL FATE

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Cellular metabolism and epigenetics play a key role in the maintenance and differentiation of embryonic stem cells (ESCs). Cells of the inner cell mass utilize glycolysis and oxidative phosphorylation (OXPHOS) to generate ATP. However, epiblast stem cells are exclusively glycolytic, which compared with OXPHOS is a less efficient means to produce ATP. In regards to

metabolic activity, extraembryonic endoderm (XEN) stem cells have not been thoroughly investigated, despite proteomic analysis suggesting increased OXPHOS activity. Since metabolic processes impact the epigenetic modifications to histones, we hypothesized that culturing cells with various metabolites favouring glycolysis or OXPHOS would influence differentiation. To address this, R1 mESCs and XEN E4 stem cells were used to recapitulate early stages in embryogenesis. Both lines were cultured in specific metabolite containing-media and assayed for metabolic activity and differentiation. Preliminary work has shown the OXPHOS profile accompanies differentiation, but is attenuated under hypoxic and conditions promoting glycolysis in F9 embryonal carcinoma cells. To corroborate these results, the metabolic profile of mESCs and XEN cells were investigated using qPCR and immunoblot analysis followed by measuring mitochondrial activity using MitoSOX and TMRM staining. Having established a baseline, mESCs were cultured in normal media, or in media containing low glucose, pyruvate, lactate or galactose, and mRNA and protein levels corresponding to metabolic enzymes involved in glycolysis and OXPHOS were measured using qPCR and immunoblot analysis, respectively. Mitochondrial activity was monitored using MitoSOX and TMRM staining, while ATP levels were determined using a bioluminescence assay. mESCs cultured in a specific metabolite would spontaneously differentiate and this efficiency was determined by qPCR and immunofluorescence. Furthermore, changes in the acetylated state of Histones 2b, 3 and 4 were assessed using immunoblot analysis, and together the results point to dynamic changes to the epigenetic landscape resulting from variations in cellular metabolism that regulate ESC differentiation.

Funding Source: This work was supported by NSERC of Canada and CHRI.

W-3056

GENERATION OF INDUCED PLURIPOTENT STEM CELLS (HIPSCS) FROM SOMATIC CELLS OF INDIVIDUALS WITH SPECIFIC PHENOTYPES FOR BLOOD TRANSFUSION

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Human induced pluripotent stem cells (hiPSCs) have great potential to become a source of RBCs, including RBCs of a particular phenotype. The objective of this work was to generate hiPSC from mononuclear cells of peripheral blood (PBMCs) from blood donors who presented low immunogenic phenotype for transfusion, and erythroid differentiation of the generated hiPSCs. Peripheral blood samples from 11 individuals were collected and characterized for the following erythrocyte antigens: Rh system (RHCE*01/RHCE*02/RHCE*03/RHCE*04/RHCE*05), Kell (KEL*01/KEL*02), Duffy (FY*01/FY*02 and FY*02N.01), Kidd (JK*01/JK*02), MNS (GYPB*03/GYPB*04). Samples (PBMC PB02 and PBMC PB12) were chosen for iPS generation due to their multiple negative erythrocyte antigens. They were isolated, expanded into erythroblasts, and transfected using the reprogramming episomal vectors PEB-C5 and PEB-Tg. This population was co-cultured on mouse embryonic fibroblasts (MEFs) until the appearance of hiPSC like colonies (hiPSC PB02 and hiPSC PB12). These colonies were transferred to human embryonic stem cells (hESCs) culture conditions and characterized regarding their pluripotency. The expression of OCT4, SOX2 and NANOG pluripotency genes demonstrated that the expression of both lineages was higher in comparison with non-pluripotent lineages. Immunophenotyping performed by flow cytometry revealed that 86% of cells expressed Nanog, 88% Oct4 and 88% Sox2. Expression levels of pluripotency genes and markers were consistent with undifferentiated state found in known pluripotent cells. Functional analysis for pluripotency was achieved by the hiPSC injection in immunodeficient mice showing that both hiPSC cell lines were able to induce teratoma tumor. The hematopoietic differentiation potential was confirmed using methylcellulose assay, with an average

of 10.5 erythroid colonies from 50x103 single cells and a mixed colonies of myeloid and lymphoid cells) and finally a colony composed of white cells from 15x103 PB12 hiPSC. In conclusion, it was possible to generate a hiPSC from a red blood cell phenotype that are negative for multiple antigens, and this cell line can be maintained for a long period in culture (26 passages) and show potential for hematopoietic differentiation.

Funding Source: CTC/FAPESP; CNPq, CAPES and FUNDHERP

W-3058

GENOME-WIDE RECESSIVE GENETIC SCREENING FOR MEIOTIC INITIATION IN GERM CELL SPECIFICATION IN VITRO MODEL

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Meiosis is a unique process that generate gametes with a haploid genome. Up to date, retinoic acid (RA) is known as a crucial signal to initiate meiosis. It activates Stra8, which has been shown to be a cytoplasmic factor to regulate pre-meiotic DNA replication, and initiate meiosis in both spermatogenesis and oogenesis. However, besides this activate signaling pathway, it is largely unknown of the repressive molecular mechanism that prevent non-germ cells and premature germ cells to enter meiosis. In our previous study, we had established process to induce meiosis in vitro from embryonic stem cells (ESCs) derived primordial germ cell like cells (PGCLCs). Here we report a genome-wide loss-of-function screening with a CRISPR-guide RNA Library on our in vitro meiosis model with a Stra8 reporter to identify genes repressing meiosis initiation. The library has 130209 guide RNAs which covers 20611 mouse protein-coding genes. We introduced Cas9 and sgRNA library into Stra8-EGFP ESCs to establish mutant library and induced it differentiating into PGCLCs. We sorted the EGFP expressing cells at ESCs and PGCLCs stage before inducing meiosis initiation and identified guide RNAs enriched in these cells. The gene sequencing result showed that 30 genes had multiple guide RNAs targeting and high copy number in both ESCs and premature male PGCLCs. Then we chose two genes to do gene knock out experiment. Knocking out these two genes could activate Stra8 expression in both ESC and PGCLC state. QPCR analysis showed that knocking out of these two genes could upregulate early meiosis stage related genes. Our study showed that it is plausible to have meiosis suppressors which also regulates in meiosis initiation.

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W-3060

ADVANCES IN DOWN SYNDROME DISEASE MODELING USING HUMAN IPSC

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Down Syndrome (DS) is one of the most frequent genetic causes of intellectual disability characterized by several pathological phenotypes; among which neurodegeneration, muscular dystrophy and cardiac defects are key features. In an effort to better understand the pathogenesis of DS, we have analysed amniotic fluid (AF) from woman carrying DS pregnancy compared with that from women carrying healthy fetuses. We have currently optimized the protocol of deriving DS iPSC from fresh DS AF samples through delivery of the Yamanaka transcription factors via non-integrating episomal vectors and compared them to iPSC derived from gestation-matched fetuses, which are karyotypically normal. These DS iPSC have in turn been characterized and differentiated to form motor neurons and cardiomyocytes. In parallel, we have carried out a targeted removal of the trisomy via clustered regularly interspaced short palindromic repeats (CRISPR) technology to create isogenic, disomic lines of the iPSC derived from DS AF cells. These DS iPSCs derived from the AF would further allow for us to study the role of DS specific biomarker genes, by comparing it with healthy closely matched controls. The use of CRISPR on these DS iPSC lines have allowed us create targeted knockouts of DS specific candidate genes and subsequently, we hope to study the effect of inhibiting these genes on cellular phenotypes derived from DS iPSC; using the reverted disomic lines as control to provide further novel mechanistic insights into AD pathogenesis. This study will lay the groundwork for understanding the effect of aneuploidy in the early development of DS and establishing DS cellular phenotypes in culture. Furthermore, it would establish the generation of efficient techniques in deriving iPSC from fetal stem cells as well as identifying possible drug targets for DS therapeutics.

Funding Source: Biomedical Sciences Institutes, A*STAR Singapore

W-3062

3D HUMAN IPSC-DERIVED HEPATOCYTES SPHEROID MODEL OPTIMIZED FOR HIGH-THROUGHPUT FUNCTIONAL ASSAYS

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Amgen, Cambridge, MA, USA

Non-human preclinical models often have poor translational value in discovery of novel drug and safety targets leading to poor efficacy and unforeseen safety liabilities upon clinical evaluation. To overcome this limitation, we have leveraged human genetics research from GWAS studies to identify natural genetic variants associated with phenotypes of interest. Models suitable to provide mechanistic insights into the underlying biology of these targets have not been fully realized. Primary human hepatocytes (PHH) are a popular system for in vitro pharmacology, toxicology and MOA studies because they faithfully recapitulate many aspects of the in vivo biology. However, genome editing tools in PHH are limited, and significant lot-to-lot variability limits the reproducibility of studies performed in this system. To overcome these limitations, we employed genome editing technology to generate hiPSC lines bearing clinically important genetic variants and optimized a differentiation protocol to yield mature hepatocytes. While 2D culture methods were only viable in large well formats, we were able to form spheroids from these cells which tolerated plating in assay-ready formats well. Finally, we demonstrated the utility of this platform to interrogate target biology with functional assays related to our targets of interest MOA studies.

W-3064

DISEASE MODELLING WITH INDUCED PLURIPOTENT STEM CELLS AND MESENCHYMAL STROMAL CELLS SHEDS LIGHT ON THE PATHOGENESIS FROM MONOGENIC OSTEOCHONDRITIS DISSECANS TO EARLY ONSET OSTEOARTHRITIS

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Osteoarthritis (OA) is a degenerative joint disease that affects millions of people globally with no disease-modifying strategies yet available. Our understanding of the pathology of OA is inadequate and this impedes investigation of efficient diagnosis and treatment. To expand our understanding of the underlying cellular pathology of OA, we studied a monogenic condition, familial osteochondritis dissecans (FOCD), associated with a known heterozygous G-> A mutation in exon 17 of ACAN gene. Patients with FOCD develop early onset OA with multiple joint involvement. The objectives of this project were to investigate the cellular pathogenesis of FOCD by studying (a) chondrogenesis of patient-derived bone marrow-mesenchymal stem cells (BM-MSCs) and (b) induced pluripotent stem cells (iPSCs) generated from patient fibroblasts. Our findings revealed that the mutation resulted in a misfolded or unfolded aggrecan protein, which accumulated in the rough endoplasmic reticulum (rER) during protein production. The consistent accumulation resulted in ER stress throughout chondrogenesis. Moreover, the rER stress caused abnormal or disregulated global extracellular matrix (ECM) production and assembly. Importantly, ECM composition analysis indicated that the patient chondrocytes produced abundant amounts of OA-associated markers. In conclusion, using patient-specific stem cell models, we have discovered a cellular pathogenesis of FOCD involving abnormal cell function and defective tissue formation, contributing to the OA phenotype.

Funding Source: This study was funded by Science Foundation Ireland Grant SFI 09/SRC.B1794 and Irish Research Council Grant GOIPG/2014/96.

W-3070

2D AND 3D DISEASE MODELING OF BECKER AND DUCHENNE'S MUSCULAR DYSTROPHIES

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Duchenne and Becker muscular dystrophies (D/BMD) are caused by a variety of mutations in the dystrophin gene and collectively comprise the most prevalent congenital skeletal muscle disorders. Although a recent therapeutic treatment has been approved by the FDA, long term therapeutic benefits are pending and subpopulations of D/BMD patients do not benefit from it. Drug discovery in D/BMD, as well as other orphan disorders, is typically hampered by the lack of adequate disease models, clinically irrelevant assays or a combination of both. Herein, we present a human pluripotent stem cell (hPSC) derived two- and three-dimensional (2D and 3D) D/BMD disease modeling platform, amenable to use with hPSCs carrying any type of dystrophin mutations. Skeletal muscle differentiation is achieved using Genea Biocells' simple three media process as previously described by Caron et al. (Stem Cell Trans Med, 2016). For 2D modeling, monolayer hPSCs are plated onto collagen I-coated dishes and differentiated through three myogenic developmental stages (e.g. satellite-like cells, myoblasts and myotubes), generating cultures suitable for morphological and metabolic analyses and cell-based drug screening assays. For 3D modeling, similar to previously published work using primary human myoblasts (Madden et al., eLife, 2015), hPSC-derived myoblasts generated in the 2D system were encapsulated in hydrogel based myobundles to promote differentiation into myotubes using a 3D culture environment. The 3D myobundles are suitable for histology, as well as contractile force measurements, both critical clinical outcome measures. Collectively, these platforms provide a patient-specific disease-in-a-dish model with clinically-relevant assay endpoints for drug discovery programs. Moreover, this approach can be extended to model a variety of other skeletal muscle disorders.

W-3072

PAF-MYC AXIS IS REQUIRED FOR STEM CELL EXPANSION IN INTESTINAL REGENERATION AND TUMORIGENESIS

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The underlying mechanisms how stem cells are conditionally expanded during tissue regeneration and tumorigenesis remain ambiguous. PAF is expressed in intestinal stem cells (ISCs) and progenitor cells and markedly upregulated during intestinal regeneration and tumorigenesis. Whereas PAF is dispensable for the intestinal homeostasis, upon tissue injury, genetic ablation of PAF leads to the impaired intestinal regeneration with the severe loss of ISCs. Mechanistically, PAF conditionally occupies and transactivates c-Myc promoter, which is essential for the expansion of ISCs during intestinal regeneration. Furthermore, PAF knockout inhibits intestinal tumorigenesis with the suppressed c-Myc expression and the reduced stemness of tumor cells in vitro and in mouse models. Collectively, our results unveil that PAF-Myc signaling axis is essential for the expansion of self-renewing cells during intestinal regeneration and tumorigenesis. 2 / 2

W-3074

MODULATION OF STEM CELL BEHAVIOR USING MONOLAYERS BINARY COLLOIDAL CRYSTALS

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The control of stem cell behavior on biomaterial surfaces is the key to a broad range of biomedical applications. The specific surface properties of biomaterials such as nanotopography and/or chemistry can profoundly influence cell morphology, proliferation, and differentiation. Recently, we established a group of elaborate surfaces that can display random and ordered micro-/nanotopographies with tunable chemistry called self-assembled monolayer binary colloidal crystals (BCCs). The BCCs were fabricated using evaporation induced colloidal self-assembly (EICSA). A library of various combinations was established. Four BCCs (5SiPM, 5SiPS, 2SiPM, and 2SiPS: particle diameter/ large particle/small particle) were selected for cell

culture. Cellular responses of human mesenchymal and pluripotent stem cells (hMSCs and hESCs) on BCCs were investigated in terms of adhesion, proliferation, and differentiation. In general, stem cells were influenced by the surface properties of BCCs resulting in a smaller spreading area compared with flat controls. Our results showed that BCCs can modulate stem cell behavior in terms of attachment, proliferation, and differentiation. In addition, cell reprogramming of fibroblasts into induced pluripotent stem cells (iPSCs) was improved using BCCs suggesting that BCCs can be used as next generation cell culture tool.

Funding Source: The Australian Research Council (ARC) are acknowledged for providing a Discovery Early Career Researcher Award (DECRA) to PYW.

W-3076

COMPARISON OF CELL CULTURE PERFORMANCE IN LARGE SCALE CLOSED MICROFLUIDIC CHIPS VS. TRADITIONAL METHODS

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ATMP (Advanced Therapy Medicinal Products) represent a new generation of treatments promising to ultimately cure many diseases including cancers, diabetes and neuro-degenerative diseases with unprecedented efficiency. However delivering ATMP implies long, complex and expansive production and quality control steps leading to expansive treatments (ranging from 50k€ to 2M€ per patient) and a difficulty to stabilize product quality. As a result both the price and the difficulty to upscale ATMP production and quality control could limit its development and the accessibility to the majority of patients. We designed an upscalable microfluidic chip that is capable of performing fundamental manipulations required for ATMP production and quality control: surface coating, cell filtration, cell seeding, medium replacement, washing, freezing, thawing and harvest. We used these chips for MSC (Mesenchymal Stem Cell) seeding, amplification, differentiation, and harvest. We compared reactant consumption and efficiency with ordinary operations in flasks using a MEF (Mouse Embryonic Fibroblast) cell line for amplification and transduction. Our results demonstrate the capability of this chip to be used as a general purpose platform in ATMP production and quality control for operations such as cell amplification, differentiation, transduction, filtering, re-concentration, incubation, washing, while providing high homogeneity, and the possibility of real time imaging. We also demonstrate a significant decrease (>90%) in reactant consumption for ordinary cell culture and transduction compared to standard protocols in performed in flasks.

W-3080

SYNCHRONIZING STEM CELL VARIABILITY TO SCREEN A POPULATION PHENOTYPE IN A HUMAN PSC POOL

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Human induced pluripotent stem cells (hiPSC) provide a promising opportunity to establish human-specific models of various diseases for drug development. Population-scale iPSC reprogramming initiatives will allow for the study of any imaginable population cohort for disease phenotypes, drug safety and efficacy. However, one to one comparison of each individual stem cell is essentially inefficient and completely unrealistic due to both time and cost considerations. Therefore we approached with a viable platform to screen a population phenotype in a pooled sample of various donor derived iPSCs by developing 3 core methods for: 1) synchronizing growth kinetic variabilities in cell maintenance, 2) coordinating differentiation of stem cell pool, and 3) decoding the individuals at single cell level. The clone-dependent proliferative variation causes preferential domination problems after several passaging among four different cell mixed culture; therefore, we sought to modulate stem cell metabolism by optimizing cultural supplements and environment. Our culture method enabled successful growth synchronization and maintenance in each cell population. Subsequently, pooled iPSCs co-differentiated directly into endoderm and mesoderm lineages with a sustained population ratio. Interestingly, FACS based quantification of the specific iPSC clone showed significant improvements in endothelial differentiation efficiency after the pooled synchronization culture. Although clone-dependent propensity to certain lineage has reported to be a major issue, this approach potentially addresses the issues of iPSC variability presumably through an epigenetic adaptation by stem cell pooling strategy. Moreover, in order to decipher individual donors from pooled cells, we carried out exon-SNPs based decoding strategy from bulk RNA-seq. The 26,794 SNPs variants set was applied to individual donor identification in iPSC pool. The transcriptome of differentiated cells from iPSC pool successfully determined the cell population. Thus, stem cell synchronization approach provides an unprecedented powerful approach for evaluating a population phenotype in a dish, leading to an enthusiastic application for conducting clinical trial in a

dish and promoting drug development process as well as precision medicine.

Funding Source: NYSCF PREST CCRF

W-3082

ENHANCED GENERATION EFFICIENCY OF HUMAN INDUCED PLURIPOTENT STEM CELLS BY SYNTHETIC PEPTIDES

Lee, Hyun[1], Lee, Dong Woo[2], Lee, Hyun Jung[1], Kim, Yeonsu[2], Park, Yoon Shin[3], Lee, Ju Yeon[2], Lee, Gene[1], Chung, Chong Pyoung[2] and Park, Yoon Jeong[1]
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The generation of human induced pluripotent stem cells (iPSCs) is useful tools for studying the pathogenesis of human disease and has the potential for clinical treatment of degenerative disease. Although the generation of iPSCs by the ectopic expression of reprogramming transcription factors (Oct4, Sox2, Klf4 and c-Myc) is groundbreaking technology, the use of integrating viral vectors still represents an obstacle for being used clinically. Therefore, alternative approaches to deliver the reprogramming factors with minimal or absence of genetic modifications are required. Here, we describe methods that use EMT (epithelial-to-mesenchymal transition) regulating peptides along with single nucleofection of reprogramming transcription factors in human dermal fibroblast. Cells are nucleofected with reprogramming transcription factors, and treated either EMT regulating peptides or control peptides daily. At day4 after nucleofection, epithelial like cells are observed in EMT regulating peptides treated group, whereas control peptide treated or non-treated group don't show any change of cellular morphology until day 10. Flow cytometry results reveal that EMT regulating peptides treated group increases epithelial marker (EPCAM, epithelial cell adhesion molecule) up to 19.38% for peptide M1 and 17.49% for peptide M2 at day 10. However, control peptide is 13.91% of EPCAM positive and non-treated group shows 12.69% of EPCAM positive. Also, EMT regulating peptides significantly increases the number of colony generated and ALP staining, compared to non-treated group or control peptide group. Furthermore, the colonies generated by nucleofection and EMT regulating peptides show strong fluorescence intensity of Nanog and Sox2 as a pluripotency marker. These colonies are successfully maintained more than 50 passages and differentiated into all three embryonic germ layers both in vitro and in vivo. Taken together, our method eliminates potential risks associated with the use of viruses, but suggests a safe and efficient way of generating iPSCs without feeder cells, providing the promising technique for patient-derived iPSCs.

Funding Source: This work was supported by the Bio & Medical Technology Development Program (#NRF-2012M3A9C6049727, 2014M3A9E3064433) of the National Research Foundation (NRF) funded by the Ministry of Science, ICT, and Future Planning.

W-3084

IPSC CELL LINE WITH ENHANCED MODULAR INTEGRATION CAPACITY

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The use of CRISPR-Cas9 systems as tools for functional genomics has become prevalent in recent years. Libraries of guide RNAs for gene knockout and gene activation/repression to conduct genetic screens have been or are currently being developed by a number of different sources. With an abundance of such tools available to the researcher, a need for standardization among cellular models is apparent, yet unrealized. With this goal in mind, we have created a system that will allow for rapid integration of Cas9 and Cas9-based effector molecules, coupled with the ability to switch from knockout to non-knockout modes of genetic regulation. This system relies on the use of donor molecules containing an appropriate arrangement of trans-recombining elements that permit rapid screening for the integration of a "landing pad" within an inert locus within the human genome. Further, this landing pad can be used as a target site for effector molecule exchange, promoter swapping, or removal of the Cas9 system entirely. This system can not only be used in any human cell type to standardize screening platforms, but can also allow for creation of modified cell lines in a more rapid and reproducible manner.

THURSDAY, JUNE 15, 2017

LATE BREAKING POSTER SESSION II-ODD 18:00 – 19:00

T-3001

SUCCINATE PROMOTES THE HUCB-MSC CYTOSKELETAL REORGANIZATION THROUGH GPR91-DEPENDENT REGULATION OF MITOCHONDRIAL DYNAMICS

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When cells are exposed to the ischemic condition, TCA cycle-involving enzyme reverse action occurs so that TCA-cycle intermediate metabolites are accumulated and among them succinate is essential for regulating the cell function. A recent study elucidated that mitochondrial pro-fission protein DRP1 controls cell migration. The physiological role of succinate in the human umbilical cord blood-derived mesenchymal stem cell (hUCB-MSC) has not yet been elucidated. In this study, we investigated the signaling pathway which is related with succinate-induced hUCB-MSC motility. Succinate (50μM) significantly accelerated hUCB-MSC migration, which was inhibited by the transfection of succinate receptor1 (gpr91) siRNA. Succinate increased phosphorylation of pan-PKC, especially atypical PKCζ level which was blocked by Gαq, Gαi, Gα12 knockdown. PKCζ subsequently phosphorylated Rho, Rac, cdc42 that were blocked by staurosporin. Succinate induced not JNK, ERK phosphorylation but p38 MAPK phosphorylation which inhibited by rho, rac, cdc42 siRNA transfection. Cytosolic DRP1 was phosphorylated by p38 MAPK so that DRP1 translocated to mitochondria outer membrane and eventually mitochondrial fission occured. Treatment of Mdivi-1 and transfected drp1 siRNA for inhibiting or decreasing the DRP1 phosphorylation resulted in decrease F-actin formation and finally hUCB-MSC migration. After succinate treatment, the ATP levels and mitochondrial membrane potential (Δψm) were increased in a time dependent manner. Concurrently, ATP level and Δψm were down-regulated by drp1 siRNA transfection. In conclusion, succinates activate PKC/Rho,Rac,cdc42/p38 activation to promote DRP1 translocation to mitochondria which result in promoting migration via F-actin formation.

T-3003

CELLULAR AGING OF ADIPOSE STEM CELLS CONTRIBUTES TO COLD INDUCED BEIGE ADIPOCYTE FAILURE IN MICE AND HUMANS

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Cold temperatures induce progenitor cells within white adipose tissue to form beige adipocytes that burn energy and generate heat, a potential anti-diabesity therapy. However, this thermogenic potential to form cold-induced beige adipocytes declines during aging. This creates a clinical roadblock to the therapeutic promise for older individuals, who constitute a large percentage of the obesity epidemic. We show aging murine and human beige progenitors display a cellular senescence-like phenotype that accounts for their age-dependent failure. Activating the senescence pathway, either genetically or pharmacologically, in young beige progenitors induced premature cellular senescence and blocked their potential to form cold-induced beige adipocytes. Conversely, genetically or pharmacologically reversing cellular aging, by targeting the p38/MAPK-p16Ink4a pathway in aged mouse or human beige progenitors, rejuvenated cold-induced beiging. This in turn increased energy expenditure and glucose sensitivity. These data indicate that anti-aging/senescence modalities could be a strategy to induce beiging and to improve metabolic health in aging humans.

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T-3005

THE STENT WITH GROWTH FACTOR SECRETING UCB-MSC ENHANCED RE-ENDOTHELIALIZATION

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Artery and vascular obstructions are known as atherosclerosis. Atherosclerosis causes plaque to form within the vasculature can eventually leads to stroke

or heart failure. Currently cardiac stenting is the most effective and a less invasive approach to treat the disease. However in-stent restenosis has been the most complex and chronic side-effect of the stenting treatment. In this study, to reduce the restenosis of stent, we used growth factor secreting stem cell coated stent. To evaluate the re-stenosis and potential therapeutic use of stem cell coated stent, we transplanted stent into the swine model. After 4 weeks of cardiac stenting treatment, we analyzed re-stenosis via Optical coherence tomography (OCT), micro-computed tomography (mCT) and immunostaining. We found controlled release of Hepatocyte growth factor (HGF) by mesenchymal stem cell successfully helped natural re-endothelialization within the stent, thus reduced the stenosis markedly. Interestingly we also found vascular endothelial growth factor (VEGF) releasing mesenchymal stem cell did not prevent re-stenosis in swine model. Our finding have significant implications for the future clinical stenting therapy. It appears that HGF secreting mesenchymal stem cell coating on stent would reduce side effects of cardiac stenting with superior re-endothelialization.

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T-3007

MEDIATING ENHANCER ACTIVITY TO CONTROL STEM CELL FUNCTION IN HEALTH AND DISEASE

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Enhancer-bound transcription factors rely on cofactors to communicate with the general transcription machinery at promoters and, thereby, establish cell-specific gene expression programs. These tightly regulated transcriptional networks control the identity of hematopoietic stem cells (HSCs) and their ability to self-renew, differentiate or transform in response to oncogenic triggers. One such crucial transcriptional cofactors is the Mediator complex (Mediator), a huge multisubunit complex that acts as a "molecular bridge" to facilitate promoter-enhancer communication. A growing amount of recent reports have identified mutations on MED12, a key member of the Mediator kinase module, in multiple blood malignancies. However, despite being a pivotal modulator of gene-expression dynamics, little is known about the role of Mediator and its individual subunits in normal and malignant hematopoiesis. We demonstrated that MED12 is an essential regulator of HSC homeostasis as in vivo deletion of MED12 causes rapid bone marrow aplasia. Deletion of other members of the Mediator kinase module did not affect HSC properties, which suggests that MED12 exerts kinase-independent functions in undifferentiated hematopoietic populations. Further mechanistic characterization revealed that MED12 is located at key HSC-enhancers, where it stabilizes the binding of the histone acetyl transferase P300. Consequently, MED12 loss perturbed enhancer activity and disrupted essential gene signatures, thus compromising HSCs survival. By analyzing HSCs and transformed cells, we show now that MED12 localizes to essential enhancers that control genes commonly altered in leukemia. Based on our results, we are exploring whether MED12 mutations perturb enhancer activity and, thereby, gene expression networks during leukemia initiation and progression. Collectively, our work sheds light on the molecular mechanisms underlying Mediator-dependent enhancer regulation in the control of physiological and malignant hematopoiesis.

T-3009

INTERFERON-GAMMA PATHWAY REGULATES EMERGENCE OF ENGRAFTABLE HEMATOPOIETIC STEM AND PROGENITOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Derivation of functional human hematopoietic stem cells (HSCs) from autologous human pluripotent stem cells (PSCs) has been an elusive goal of treatment of hematologic disorders and malignancy. Building upon recent evidence that HSCs are derived from definitive hemogenic endothelium (HE), we identified 7TFs (ERG, HOXA5, HOXA9, HOXA10, LCOR, RUNX1, SPI1) that were sufficient to convert human PSC-derived HE into engraftable hematopoietic stem and progenitor cells (HSPCs) with long-term and multilineage capacity (HE-7TF cells, Sugimura, Nature in press). In the current study, we attempted to define signaling pathways that further enhance engraftment of HE-7TF cells. We

investigated the source of our long-term engraftable cells by sorting CD34+CD43+CD45+ triple positive cells and CD34+CD43-CD45- single positive cells from HE-7TF cells. We transplanted these two cell populations into non-irradiated c-Kit deficient immune-deficient recipients, and monitored engraftment capacity after 8 weeks of transplantation. 2 out of 5 mice transplanted with triple positive cells showed multi-lineage engraftment, whereas 5 mice transplanted with single positive cells did not, suggesting that CD34+CD43+CD45+ triple positive cells are the source of long-term engraftment in our system. We then investigated what signaling pathways regulate the emergence of CD34+CD43+CD45+ triple positive cells from HE. From high-throughput screening of 2,000 receptor ligands, cytokines/morphogens, hormone, and chemical compounds, we observed that interferon-gamma (IFNγ) increased CD34+CD43+CD45+ triple positive cells 6 to 8-fold in 5 independent experiments. The effect was abrogated by the addition of IFNα. Collectively, these data demonstrate that the IFNγ pathway promotes the emergence of engraftable HSPCs from human PSC-derived HE.

T-3011

COMPLEMENT DEPLETION IMPROVES HUMAN RED BLOOD CELL RECONSTITUTION IN IMMUNODEFICIENT MICE

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The recent development of novel gene editing and stem cell technologies has increased the potential to cure hematological disorders, but the lack of suitable animal models that faithfully simulate human hematological physiology and pathology has hampered their clinical translation. We have shown that human red blood cells (hRBCs) are subject to robust rejection by mouse macrophages in immunodeficient mice. In this study, we found that mouse serum induces hRBC adherence to murine phagocytic cells, including professional phagocytic macrophages and neutrophils and non-professional phagocytic cells, such as endothelial cells. Complement is responsible for mouse serum-induced hRBC adherence to murine phagocytic cells, as complement-depleted mouse serum lost its ability to induce hRBC adherence to murine phagocytes. Although hRBC survival was not improved in NOD/SCID mice that were treated with cobra venom factor (CVF) to deplete complement, CVF significantly prolonged hRBC survival in mice that were depleted of phagocytic macrophages by clodronate-liposomes. This combination treatment also synergistically improved hRBC reconstitution in human CD34+ cell-grafted mice. These data indicate that complement, which might be dispensable for hRBC rejection by murine phagocytic macrophages, is critical in hRBC rejection by other types of murine phagocytic cells, such as neutrophils and endothelial cells. Further, combination treatment with clodronate-liposomes and CVF is a simple approach for improving hRBC reconstitution in immunodeficient mice.

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T-3013

EFFECT OF CHRONIC LUNG INJURY IN THE REGENERATIVE CAPACITY OF ENDOGENOUS MOUSE LUNG STEM CELLS

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Idiopathic Pulmonary Fibrosis (IPF) is the most common and aggressive form of idiopathic interstitial pneumonias. The prevalence and mortality of IPF has been increasing in recent years. The pathogenic mechanisms are poorly understood, in the last decade, however, it has been proposed that IPF probably results from multiple cycles of epithelial cell injury and activation that in consequence induce proliferation, and activation of mesenchymal cells, leading to the exaggerated accumulation of extracellular matrix (ECM). Type 2 alveolar epithelial cells (AEC2) are the progenitor cells of the alveolar region. The mechanism by which impaired AEC2 function leads to fibrosis is not understood. We hypothesize that chronic alveolar epithelial damage leads to altered lung stem cell functions. We performed experiments to determine how lung stem cell function is impacted in an established repetitive bleomycin injury model of pulmonary fibrosis, in which up to 8 doses of bleomycin were administered with intermittent time points for epithelial cell response and recovery prior to re-injury. Our preliminary results show by FACS, a significant increase in the percentage of EPCAM +/ SCA-1+ distal epithelial progenitor cells

and a decrease of the EPCAM+/SCA-1- (AEC2 enriched) population in cells obtained from bleomycin-injured lungs compared with their PBS controls ($p < 0.05$). Using a 3D co-culture assay that allows us to quantitatively compare differentiation and proliferation potential of lung progenitor cells, we observed that both EPCAM+/SCA-1+ and EPCAM+/SCA-1- populations from bleomycin treated lungs, show a significant decrease in colony forming efficiency compared with controls ($p < 0.01$) and increased differentiation towards the alveolar lineage in the EPCAM+/SCA-1+ progenitors compared with control lungs ($p < 0.05$). These results suggest that chronic lung epithelial cell injury impairs the proliferation and differentiation capacity of endogenous lung stem cells. Ongoing studies will define the mechanisms of these effects, which may reveal new therapeutic targets for IPF. A link between IPF and any one of these effects adult lung progenitor cells will bring fresh insight on the mechanisms of pulmonary fibrosis and reveal new therapeutic targets.

Funding Source: Mexico's Council of Science and Technology/Mexico in Harvard Foundation Fellowship

T-3015

EXTRACELLULAR MATRIX MICROENVIRONMENT ENHANCES NEURONAL DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human induced pluripotent stem cells (hiPSCs) reprogrammed from human somatic cells by delivering defined transcriptional factors not only provide therapeutic cell sources for neuronal regeneration, but also biomedical platforms for drug screening and in vitro disease modeling of neuronal disorders. Despite the advances in differentiation protocols, enhanced neuronal differentiation of hiPSCs still remains a challenge. Here we report an extracellular matrix (ECM)-based microenvironment system to provide biochemical and biophysical cues for promoting neuronal differentiation of hiPSCs in vitro. Three-dimensional (3D) ECM hydrogel significantly facilitated neuronal lineage differentiation of hiPSCs as confirmed by upregulated expression of several neuronal markers and highly matured neuronal morphologies. In this study, we demonstrate that reconstituted ECM microenvironment could provide an efficient culture platform to produce functional neuronal lineage cells derived from hiPSCs.

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T-3017

RHOA REGULATES HIPPO MEDIATED E-REG SIGNALING TO CONTROL INTESTINAL STEM CELL REGENERATION

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RhoA GTPase has multiple cell regulatory functions throughout development. To determine the physiological function of RhoA in small intestine stem cells (ISCs), we utilize a Villin-CreERT2;rhoA flox/flox mouse genetic model to inducibly delete RhoA in small intestinal epithelium. Loss of RhoA caused a loss of epithelia polarity, a severe defect in adhesion junction, and mis-localization of Paneth cells. RhoA knockout mice also exhibited reduced proliferation and elevated apoptosis in crypts, a loss of ISCs, and differentiation defects that mimic effects of radiation damage. Enteroid culture studies revealed a requirement of RhoA for enteroid growth and ISC regeneration. Mechanistically, RhoA deletion resulted in reduced Hippo-Yap signaling and its effector E-reg expression in the crypts. Expression of a stabilized Yap allele could readily rescue ISC marker expression, ISC regeneration and ISC-associated Wnt signaling in RhoA knockout mice, indicating that Yap acts downstream of RhoA for ISC function. Finally E-reg treatment rescued the enteroid growth phenotype of RhoA null ISCs. Our studies implicate RhoA mediated Yap-Ereg signaling as a key regulator of intestine homeostasis and ISC regeneration.

T-3019

SELECTIVE ABLATION OF TUMORIGENIC CELLS FOLLOWING HUMAN IPSC-NS/PC TRANSPLANTATION IN SPINAL CORD INJURY - THE USE OF SUICIDE GENES IN STEM CELL THERAPY

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The issue of safety is one of the highest concerns when it comes to the clinical application of hiPS-NS/PC transplantation in treating spinal cord injury (SCI).

With certain "tumorigenic cell lines" transplanted into murine SCI models, there is an initial improvement in motor function followed by an abrupt deterioration secondary to the mass effect of the tumor. A significant proportion of these cells remain undifferentiated post transplantation. The aim of this study is to selectively ablate these undifferentiated cells whilst preserving the differentiated cells and hence the motor function. Herpes Simplex Virus 1 Thymidine Kinase (HSV-TK) gene is a well-known suicide gene used in the clinical setting. Ganciclovir (GCV), the prodrug of HSV-TK, can be converted to cytotoxic GCV-triphosphate by HSV-TK, thereby killing HSV-TK-expressing cells. It is cell cycle dependent. We lentivirally introduced the HSV-TK gene into a known tumorigenic line of hiPSC-NS/PCs (hiPSC-NS/PC-HSVTK). We allowed these cells to differentiate and, following GCV administration, we observed a significant decrease in the number of immature Nestin and Ki67 positive cells (60.0% to 18.6%, 30.0% to 3.1% respectively, $p < 0.01$), while the Tuj1 positive cells were relatively preserved (84.5% to 63.3%, $p>0.05$) (n=100-120cells, experiment repeated twice). The hiPSC-NS/PC-HSVTK were transplanted into SCI model mice. GCV was administered 6 weeks following transplantation. Motor function was evaluated through weekly BMS scoring together with Rotor Rod and Digigait analysis 12 weeks following transplantation. Compared to the PBS group (n=8), only the mice in the group with GCV administration (GCV(+), n=8) showed significant improvements in motor function ($p < 0.01$). Immunohistochemistry revealed that the immature Nestin, SOX1 and Ki67 positive cells were more abundant in the GCV non-administered (GCV(-) n=8) mice (45.6%, 32%, 15.4% respectively) compared to the GCV(+) mice (4.3%, 2.2%, 1.0% respectively)($p < 0.01$). There were no significant differences in the percentage of NeuN positive cells between the 2 groups (19.5% to 23%, $p>0.05$). We were successful in selectively ablating the immature potentially tumorigenic iPS-NS/PCs that had been transplanted into SCI model mice whilst preserving the motor function gained from the treatment.

T-3021

ISCHEMIC INJURY CHANGES THE CHROMATIN LANDSCAPE OF ENDOGENOUS NEURAL STEM CELLS IN THE ADULT BRAIN

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Regenerative processes in adult tissue require re-activation of developmental programs to enable a proper tissue repair. This is the case in adult organism such as the Zebrafish. However, the adult mammalian brain exhibits a very poor regenerative capacity, owing in part to the failure in activating a regenerative program. To address the reasons underlying the failure to repair the adult brain, we examined the reaction to an ischemic injury of endogenous neural stem cells (NSCs) within the natural environment of the brain. To this end, we investigated the cellular and molecular responses of endogenous NSCs to ischemic injury. By combining multilevel in vivo lineage tracing, transcriptome analysis and tagmentation-based whole genome bisulfite sequencing (TWGBS), we identified interferons as factors involved in activation of endogenous NSC to exit the quiescent state. Furthermore, we demonstrate that ischemic injury leads to activation of an inflammatory response not only in adult NSCs but also in the surrounding oligodendrocytes and neuroblasts. Excitingly, we found that the ischemic injury induces distinct changes in the chromatin landscape in a cell-type specific manner. By using lineage tracing we further demonstrated that ischemic injury modified the migratory behavior of endogenous NSCs re-directing them to migrate towards the injury site. Altogether, our study characterizes the response to an ischemic injury of endogenous adult NSCs and uncovers some features potentially involved in the inability to repair the brain.

T-3023

HUMAN CELL MODELS OF TSC1/HAMARTIN HAPLOINSUFFICIENCY LINK MTOR PATHWAY TO DEFECTIVE TAU METABOLISM

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Frontotemporal lobar degeneration (FTLD) is a complex neurodegenerative disorder considered the second most common cause of early-onset dementia after Alzheimer's disease. Patients present with alterations in behavior or language associated with the atrophy of frontal and temporal lobes of the brain. The pathological hallmark of the disease is the presence of cytoplasmic inclusions in affected neurons containing aberrant forms of tau (a microtubule-binding protein) or TDP-43 (transactive response DNA-binding protein 43 KDa) proteins. Despite the identification of multiple genetic causes for FTLD, the molecular alterations underling the disease remain incompletely understood. We have previously identified a loss-of-function mutation in TSC1, a gene previously implicated in juvenile-onset tuberous sclerosis, as a potential cause for FTLD. TSC1 encodes hamartin, a protein that form a dimer with tuberin inhibiting mTOR activity; therefore, hamartin deficiency results in mTOR overactivation with downstream consequences including increased protein synthesis and decreased lysosome biogenesis and autophagy.

To assess how TSC1 mutations leads to FTLD, we have used cell models of TSC1/hamartin haploinsufficiency including CRISPR engineered neuroblastoma SH-SY5Y cells, fibroblasts and iPSC (induced Pluripotent Stem Cells) from a TSC1 mutation carrier. Under culture, our results show that cells containing lower levels of hamartin exhibit enhanced mTOR activity and enlargement of the cell body. After neuronal differentiation, we saw that TSC1 +/- cells showed elevated tau and phospho-tau protein levels, while TDP-43 levels were unchanged. These findings suggest a specific tau clearance impairment associated with hamartin haploinsufficiency. This novel link between mTOR signaling and tau metabolism suggests a potentially treatable mechanism for age-associated, tau-related neurodegeneration.

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T-3025

TREATMENT OF EXOSOMES FROM ADIPOSE-DERIVED STEM CELLS FOR NEURODEGENERATIVE DISEASES

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Adipose-derived stem cells (ASCs) have a potential for use in the treatment of incurable disorders, including neurodegenerative diseases. ASCs secrete various factors which can modulate a hostile environment, called paracrine effect. Exosomes are small extracellular vesicles containing cell derived factors and mediate paracrine effect of cells. Thus, exosomes from ASCs (ASC-exo) can be a potential candidate of therapeutic effects of stem cells. To investigate the effect of ASC-exo on neurodegenerative diseases such as Amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD), we used neuronal stem cells (NSCs), which can be differentiated into neuronal cells, isolated from G93A ALS or R6/2 HD mouse model. And we investigated phospho-CREB/CREB ratio, PGC-1α expression level and inclusions of abnormal proteins, such as SOD1 and mutant huntingtin, since ALS and HD have common phenotypes inculding mitochondria dysfunction and gain of function by abnormal proteins. As a result, ASC-exo treatment up-regulates PGC-1, phospho-CREB and ameliorates inclusions of SOD1 or mutant huntingtin in vitro ALS or HD models. These findings suggest that ASC-exo has a therapeutic potential for treating neurodegenerative diseases by improving mitochondrial functions and inclusions of abnormal proteins.

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T-3027

HO-1 INHIBITS INFLAMMATORY CYTOKINES WHICH FAVOR BDNF INDUCED NEUROREGENERATION IN SPINAL CORD INJURY OF DOGS AND IMPROVES FUNCTIONAL RECOVERY

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Abundant expression of proinflammatory cytokines after a spinal cord injury (SCI) creates an inhibitory microenvironment for neuroregeneration. We potentiated the function of adipose derived mesenchymal stem cells (Ad-MSCs) by transfecting them with brain derived neurotrophic factor (BDNF) and heme oxygenase-1 (HO-1), through a lentivirus. We injected both HO-1 and BDNF-overexpressed MSCs, as a combination group, to selectively control inflammation and induce neuroregeneration in SCI dogs, and compared this with BDNF MSCs, HO-1 MSCs, and GFP-MSCs injected dogs. The groups were compared in terms of improvement in BBB score during 8 weeks, western blot analysis, immunofluorescent staining, and hematoxylin and eosin (H & E) staining. The combination group showed a significant improvement in hindlimb functions, with a higher BBB score, and a robust increase in neuroregeneration, depicted by a higher expression of Tuj-1, NF-M, and GAP-43 due to a decreased expression of the inflammatory markers IL-6 and TNF-α, and an increased expression of IL-10 ($p \leq 0.05$). H & E staining showed more reduced intraparenchymal fibrosis in combination group, than in other groups ($p \leq 0.05$). Increased neuroregeneration indicates a favorable outcome of inflammatory inhibition. It was thus suggested that HO-1 MSCs reduce inflammation, which favors BDNF-induced neuroregeneration in SCI.

T-3029

CD34 POSITIVE CANCER STEM-LIKE CELLS DRIVE UV INDUCED SKIN CANCER GROWTH IN MICE

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Cancer stem cells are being recognized as initiators of various epithelial cancers including skin squamous cell carcinoma (SCC). The UV-induced SCC consists of different cell populations. In this population cancer initiating cells/ cancer stem-like cells have the potential of being drug resistance and can give rise to metastasis. In the present study we aimed to identify UV-induced cancer initiating cells. We could show that CD34+a6+ cancer initiating cells within UV-induced SCC can initiate secondary and third tumors with small number of cells. These cancer stem-like cells displayed higher tumor initiating ability, greater in vitro self-renewal ability, and higher level of nanog expression as detected by quantitative RT-PCR compared to CD34- a6+ cancer cells. Co-immunostaining of BrdU-pulsed tumor tissues revealed a majority of the BrdU positive cells are CD34 negative suggesting a possible shift from CD34+ to CD34- cells when CD34+ stem-like cells start to "selfrenew" during in vivo tumor growth stage. Detailed understanding of the molecular and cellular function of CD34 + a6+ cancer initiating cells will likely contribute to effective medical therapies in the treatment of SCC.

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T-3031

SUPPRESSION OF CAF-1 HISTONE CHAPERONE DRIVES MYELOID PROGENITOR CELL DIFFERENTIATION

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During hematopoiesis stem and progenitor cells become progressively restricted in their potential to differentiate into numerous blood cell types. This process is driven by lineage-specific transcriptional programs that are accompanied by dynamic changes of chromatin structure and associated modifications. However, the mechanisms underlying these changes of chromatin state and their roles in hematopoiesis remain incompletely understood. Recent work from our lab has uncovered a role for the chromatin assembly factor complex CAF-1 in safeguarding cell identity during cellular reprogramming to pluripotency. We demonstrated that CAF-1 stabilizes cell fate by regulating nucleosome assembly and the maintenance of heterochromatin. Moreover, we showed that CAF-1 suppression facilitates transdifferentiation of preB lymphocytes into mature macrophages, suggesting that reduced CAF-1 levels endow cells with a developmentally more plastic state. To test whether CAF-1 may also play a role during hematopoietic differentiation, we investigated the phenotypic and molecular consequences of CAF-1 depletion in a well-established paradigm of myeloid differentiation. We find that CAF-1 suppression triggers rapid differentiation of myeloid progenitors into macrophages and neutrophils, despite the presence of culture conditions that otherwise promote propagation of undifferentiated cells. We will present transcriptional and chromatin changes that drive myeloid differentiation upon perturbation of CAF-1. Together, our findings suggest that manipulation of chromatin accessibility through modulating the expression of CAF-1 provides a potential strategy for improving differentiation therapy of myeloid malignancies.

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T-3033

METABOLIC AND EPIGENETIC REGULATION OF MESC MAINTENANCE AND EMBRYONIC DEVELOPMENT BY SIRT1

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The methionine metabolism is critical for epigenetic maintenance, redox homeostasis, and animal development. However, the regulation of methionine metabolism remains undefined. Here we provide evidence that SIRT1, the most conserved mammalian NAD+-dependent protein deacetylase, is critically involved in modulating methionine metabolism thereby impacting maintenance of mouse embryonic stem cells (mESCs) and subsequent embryogenesis. We demonstrate that SIRT1 deficient mESCs are hypersensitive to methionine restriction/depletion-induced differentiation and apoptosis, primarily due to a reduced conversion of methionine to S-adenosylmethionine. This reduction markedly decreases methylation levels of histones,

resulting in dramatic alterations of gene expression profiles. Mechanistically, we show that the enzyme converting methionine to S-adenosylmethionine in mESCs, methionine adenosyltransferase 2A (MAT2A), is under control of Myc. SIRT1 modulates methionine metabolism in part through Myc-mediated expression of MAT2A. Importantly, SIRT1 KO embryos are sensitive to maternal methionine restriction-induced lethality, whereas maternal methionine supplementation increases the survival of SIRT1 KO newborn mice. Our findings uncover a novel regulatory mechanism for methionine metabolism, and highlight the importance of methionine metabolism in SIRT1-mediated mESC maintenance and embryonic development.

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T-3035

IN VITRO 3D SKIN ORGANOID MODEL FROM SYSTEMIC SCLEROSIS PATIENT DERIVED INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) are a promising unlimited cell source that can be used in disease modeling and drug screening. Patient-specific iPSCs may reflect the disease mechanism. Systemic sclerosis (SSc) is a very rare and chronic autoimmune disease. SSc is an autoimmune disease characterized by vascular damage and skin fibrosis. Skin fibrosis especially affects whole body in SSc. The pathophysiology of SSc not yet is well known. We generated iPSCs from SSc patients to study disease mechanisms. SSc patient-derived iPSCs were differentiated into each cell types of keratinocytes and fibroblasts. Skin organoid was built from 3D culture of iPS-derived keratinocyte and fibroblast. SSc-iPSCs derived 3D skin organoid revealed disease phenotype of SSc. Histologically, SSc-derived 3D skin organoid is thicker than health control. We compared gene expression pattern of extracellular matrix between health iPSC and SSc-iPSC derived 3D skin organoids. The expression level of extracellular matrix related gene was increased in SSc-iPSC derived 3D skin organoid. This result showed that differentiated cell derived from patient-specific iPSC has the peculiar phenotype. Patient-iPSCs derived 3D skin organoid can be used for disease modeling and drug screening platform.

T-3037

SHARED EFFECTS OF DISC1 DISRUPTION AND ELEVATED WNT SIGNALING IN HUMAN CEREBRAL ORGANOIDS

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The development of three-dimensional culture methods has allowed for the study of developing cortical morphology in human cells. This provides a new tool to study the neurodevelopmental consequences of disease-associated mutations. Here, we study the effects of isogenic DISC1 mutation in cerebral organoids. DISC1 has been implicated in psychiatric disease based on genetic studies, including its interruption by a balanced translocation that increases the risk of major mental illness. Isogenic wild-type and DISC1-disrupted human induced pluripotent stem cells were used to generate cerebral organoids, which were then examined for morphology and gene expression. We show that DISC1-mutant cerebral organoids display disorganized structural morphology, which is phenocopied by WNT agonism. Further studies with iPSC-derived neurons reveal that there are many shared changes in gene expression with DISC1 disruption and WNT agonism, including in neural progenitor and cell fate markers, regulators of neuronal migration, and interneuron markers. These shared gene expression changes suggest mechanisms for the observed morphologic dysregulation with DISC1 disruption and open up new avenues for future studies. The shared changes in 3-dimensional cerebral organoid morphology and gene expression with DISC1 interruption and WNT agonism further strengthens the link between DISC1 mutation, abnormalities in WNT signaling, and neuropsychiatric disease.

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T-3039

A NOVEL TOOL FOR THE SUSPENSION CULTURE OF INDUCED PLURIPOTENT STEM CELLS: LYSOPHOSPHOLIPIDS ACT AS A CELL-AGGREGATION REGULATOR OF INDUCED PLURIPOTENT STEM CELLS

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Suspension culture system is expected to be a useful tool for the mass production of human induced pluripotent stem cells (hPSCs). This production will enable the realization of the clinical applications for regenerative medicine. However, hPSCs are known as being sensitive to high shear stress that may cause unexpected cell death and/or differentiation. Therefore, suspension culture for the increase in hPSCs has been one of the major challenges. Previously, it was reported that albumin-associated lipids prevented aggregation of hPSCs (Horiguchi et al., 2016), whereas, lipids responsible for this function were unclear. Here, by using cell aggregation assay, we investigated principal lipids regulated aggregation size of hPSCs. hPSCs were dissociated into a single cell suspension. Then these cells were seeded on a multi-well culture plate with candidate lipids and incubated overnight by shaking gently. The next day, a lipid involved in the inhibition of cell-cell adhesion was determined by the aggregation size. As a result, lysophosphatidic acid (LPA) and Sphingosine-1-phosphate (S1P), known as lysophospholipids acting as a signaling molecule, were identified. These lipids regulated the aggregation size in a dose-dependent manner. Aggregates formed with these lipids kept the high-expression rates of pluripotent marker genes and had the abilities of proliferation and differentiation. These studies demonstrated that LPA and S1P were useful for suspension culture and enabled the reduction of shear stress without affecting the viability of hPSCs. This was led the development of a simple and robust method for the suspension culture of hPSCs. These findings are suggested to be a break-through technology for the large-scale and cost-effective production of hPSCs for regenerative medicine.

Funding Source: This research was supported by Research Center Network for Realization of Regenerative Medicine, Japan Agency for Medical Research and Development (AMED)

T-3041

ATTITUDES TOWARD THE CREATION AND USE OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED GAMETES: A SURVEY OF THE GENERAL POPULATION IN JAPAN

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Research for developing human induced pluripotent stem cell (hiPSC)-derived gametes is ongoing in several laboratories worldwide. Current regulations for the creation and use of human embryos using hiPSC-derived gametes vary between countries. In the US, federal laws do not explicitly prohibit the creation of human embryos for research; the NIH does not fund either the creation or fertilization of hiPSC-derived gametes whereas California funds both. In the UK, both the creation and fertilization of hiPSC-derived gametes are allowed for research purposes. In Japan, the Ministry of Education, Culture, Sports, Science and Technology approved, in December 2008, the production of gametes from human pluripotent stem cells for basic research, though the fertilization of gametes was banned. In 2013, an Expert Panel on Bioethics at the Japan Cabinet Office began examining new guidelines for germ cells derived from human pluripotent stem cells in response to research developments and social changes. In 2015, the panel reported that various opinions should be gathered from the general population before new guidelines are provided. However, attitudes of the general population toward fertilization using gametes derived from hiPSCs remain unclear. Presently, we are preparing an online survey to assess the acceptability of basic research and clinical applications involving hiPSC-derived gametes. For the Japanese public, the questionnaire provides background information on iPSCs as well as on the research using hiPSC-derived gametes. It also illustrates the purposes and processes of the research, which is divided into three steps (creation, fertilization, and gestation). The survey will ask respondents about their acceptance (for example, “up to what extent would you accept this research?”). Based on sample size calculation, we aim to include 2,832 respondents from the general public. Data will be collected upon approval from the joint medical ethical committee of Kyoto University. The results will help policymakers better understand attitudes toward the creation and use of hiPSC-derived gametes for research and reproductive purposes. In this poster presentation, we will present our research findings.

Funding Source: This research was founded by the Uehiro Foundation on Ethics and Education.

T-3043

CHARACTERIZATION OF TISSUE ENGINEERED GRAFTS CARRYING MESENCHYMAL STEM CELLS TO SUPPORT ESOPHAGEAL REGENERATION.

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Implanted synthetic scaffolds carrying autologous adipose-derived mesenchymal stem cells (aMSCs) support regeneration of the esophagus after circumferential resection. Here, we demonstrate the reproducible characteristics of aMSCs from 23 pigs on more than 70 electrospun scaffolds. The scaffolds consistently carried viable, motile porcine aMSCs. Human aMSCs from 2 individuals exhibited similar characteristics. Proteomic and quantitative polymerase chain reaction studies were performed to establish hypotheses regarding potency and mechanism of action. The resultant molecular signatures of the aMSCs highlight inflammatory and angiogenic pathways in vitro that are relevant to the proposed mechanism of esophageal growth in vivo. Pathway components including VEGFA, IL6, IL8 and MMP2 were confirmed by enzyme linked immunosorbent assay from conditioned medium. To examine whether the levels of growth factors and cytokines were within a physiological range, we used in vitro assays of inflammation and angiogenesis to test conditioned medium. First, aMSCs reduced the expression of tumor necrosis factor alpha by splenocytes primed with lipopolysaccharide. Second, conditioned medium from aMSCs grown on the scaffold accelerated the alignment of human umbilical vein endothelial cells in vitro. In summary, we demonstrate that aMSCs carried by a synthetic scaffold can provide combinations of growth factors and cytokines that may support the capacity of the esophagus to grow and regenerate. Further studies are ongoing to determine the function of the growth factors and cytokines and the ability of the implanted aMSCs to contribute to the esophagus.

T-3045

DIFFERENTIAL ACTIVATION OF THE MAPK SIGNALING PATHWAY CONTROLS MALE GERMLINE STEM CELL FATE

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Cell-to-cell competition leading to clonal selection among spermatogonial stem cells (SSCs) has been proposed to account for the accumulation of pathogenic mutations in the testes of older men and the increased incidence of disorders in children, referred to as the paternal age effect (PAE). The SSCs require a specific set of growth factors, including GDNF and FGF2, but it is controversial how FGF2 contributes to SSC maintenance. We have demonstrated that SSCs expressing the Apert syndrome PAE-associated mutation in FGFR2 (AS SSCs) exhibit enhanced competitiveness, contingent upon growth factor dose. AS SSCs exhibited increased sensitivity to FGF2 manifested by enhanced MAPK signaling and proliferated in suboptimal GDNF and FGF2 conditions, suggesting that elevated FGF2 signaling preserves stem cell activity when growth factors are scarce and that the MAPK pathway plays a central role in acquired competitiveness. Long-term culture of SSCs requires FGF2 for expansion over time in a concentration-dependent manner and several studies have shown FGF2-dependent SSC self-renewal. Intriguingly, there is also in vitro and in vivo evidence of inhibited self-renewal capacity proportional to FGF2 concentration. Moreover, most PAE mutations consist of weak gain-of-function mutations in the FGFR/RAS/MAPK pathway. Here, we address the detailed FGF2-mediated signaling mechanisms in SSCs. In contrast to GDNF, FGF2 induces a biphasic activation of MAPK such that the strongest phosphorylation of the pathway is detected at lower FGF2 doses, which also induces sustained MAPK activation. We have modeled FGF2 signaling conditions that render opposing effects on SSCs. In our experimental model, FGF2 dosage inversely correlates with colonization activity and dictates the response to retinoic acid, a SSC differentiation driver in vivo and in vitro. We have also identified the FGF2-dependent negative feedback mechanism that regulates MAPK signaling, a function we found to be critical for stem cell maintenance, as demonstrated by specific shRNA-mediated knockdown. We propose that differential MAPK activation is a molecular switch controlling SSC fate. Our data shed light on how pathogenic mutations accumulate and may explain the limited spectrum of such mutations that are compatible with positive selection in the testis.

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T-3049

ASSESSMENT OF THE APPEARANCE OF SPONTANEOUS GENETIC MUTATIONS IN HUMAN INDUCED PLURIPOTENT STEM CELLS

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Somatic mutations are thought to contribute to tumorigenicity or immortalization of normal cells. Recent studies indicate that human-induced pluripotent stem cells (iPS cells) have DNA changes involving a wide variety of somatic mutations in coding regions. Therefore, a better understanding of genetic basis of iPS cell-based products is expected to benefit their assessments of safety or efficacy in future regenerative medicine. However, it is controversial whether these mutations may have been introduced during the reprogramming process, either due to replication errors in culture or due to clonal expansion of pre-existing somatic mutations in iPS source cells. In this study, to monitor the patterns of somatic mutation during long-term culture in human iPS cells, we evaluated the number of single nucleotide variants (SNVs) during human iPS cell passaging by whole exome sequencing. The raw data of 15, 30, 40 Gb per exome from iPS cells collected at every 3 passages (until 15 passages) was mapped to the haploid human reference sequence hg 19 (GRCh37). To identify somatic mutations, we utilize a somatic SNV calling algorithms (Strelka) for each exome sequencing data, and focused on the number of SNVs, not the type of SNVs. As a result, the number of SNVs at more than 8-9% allele frequency was no significant difference in all sequence data volumes (15, 30, 40 Gb). A comparison of the number of SNVs in iPS cells at every 3 passages revealed a stable correlation among them. Interestingly, it was observed that these number SNVs in iPS cells were greater than that of other normal somatic stem cell lines or cancer cell lines. Furthermore, we also found a few protein coding mutations accumulated during passaging of iPS cells, suggesting that they possibly involve the acquisition of growth advantage. Our results suggest that the accurate determination of the frequency of spontaneous mutation appearance during cell culture provides strong insights into phenotypic changes in a cell population, which would be useful for establishment, quality control, storage and distribution of iPS cell banks/stocks, as well as for our understanding of these processes.

T-3051

SMALL-MOLECULE COCKTAILS INDUCE THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TOWARDS FUNCTIONAL HEPATOCYTE-LIKE CELLS

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Human pluripotent stem cells-derived hepatocytes provide unlimited resources for cell-therapy, pharmaceutical applications as well as for studies of developmental biology. Previously, most of efforts have been invested into the utilization of growth factors to generate hepatocytes in vitro conditions. However, some certain drawbacks hindered the broad usage of growth factors for reasons associated with stability and quality. Meanwhile, the use of small molecules to chemically direct pluripotent stem cells towards functional hepatocytes represents a much more powerful strategy due to their stability, cell-permeability, rapid onset and cost-effectiveness, which will be beneficial for scaling-up differentiation for future applications in pharmacology industry and clinical medicine. We have designed such small molecule cocktails for stepwise differentiation of human pluripotent stem cells into definitive endoderm cells and then towards functional hepatocytes. The final differentiated cells are not only morphologically similar to hepatocytes derived from growth factors-induced methods and primary hepatocytes, but also demonstrate hepatic specific markers at the transcriptional and protein levels, as well as capable of producing albumin, storing glycogen. Conclusively, by recapitulating some certain key events of liver organogenesis, we have established a cost-effective chemical defined differentiation protocol to stepwise generate hepatocytes from multiple pluripotent stem cell lines by using small-molecule cocktails.

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T-3053

SIMPLE AND SMALL-SCALE SUSPENSION DIALYSIS CULTURE FOR ENDODERMAL DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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Endodermal differentiation of the human induced pluripotent stem cells (hiPSC) have a great potential for regenerative medicine and pharmaceutical application targeting to digestive system organs. To enbale these applications, efficient large-scale culture is necessary, but it needs very high costs because the differentiation process requires a variety of growth factors at high concentrations. Dialysis culture is one of the solutions to reduce the costs from the standpoint of cell culture engineering. A dialysis culture system allows continuous nutrient supplies and waste removals by the dialysis compartment, while holding both exogenous and endogenous growth factors in the cell compartments. The feasibility of such dialysis culture was already investigated in hiPSC expansion in the stirred-suspension bioreactor, but it has not yet been applied to hiPSC differentiation where the cost of growth factors is much higher. We therefore developed a simple and small-scale suspension dialysis culture system that is suitable to examine various cultural conditions. The dialysis membrane (MWCO, 13,000) was fixed at the bottom of a commercially-available culture insert and the cup was set into the deep well plate. The dialysis cup-loaded plates were put on a rotational shaker to enable suspension culture of iPSC aggregates in the dialysis cup. The cell culture compartment was dialyzed against the lower plates (dialysis culture medium compartment). The endodermal differentiation medium was prepared by adding growth factors larger than the molecular weight cut-off of dialysis membrane into the dialysis culture medium. The volume of dialysis culture medium was six times larger than that of differentiation medium. The morphology of differentiated aggregate after five days of culture had no difference between with and without dialysis. However, the aggregates cultured with dialysis showed reduced pluripotency and proceeded to the endodermal lineage by dialysis, according to their gene expression level. Although detailed analyses of the culture medium should be done, such dialysis culture surely accelerate endodermal differentiation, thus, leading to reduction of the cost. In addition, each differentiation step can be optimized using such small-scale suspension dialysis culture system.

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T-3055

SYNCHRONIZATION OF GABAERGIC INHIBITORY INTERNEURON DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS (PSCS)

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GABAergic inhibitory interneurons comprise about 20% of total neurons in adult brains, and their dysfunction is associated with neuropsychiatric disorders such as epilepsy, Schizophrenia and Autism. Pluripotent stem cell (PSC) technology provide a platform to generate unlimited quantity of human neurons for disease modeling, drug screening as well as regenerative medicine. However, human PSC-derived progenies tend to be heterogeneous and asynchronous, with the presence of both proliferating progenitors and postmitotic neurons for extended period time, similar to in vivo embryonic development. This heterogeneity not only hinders reliable and reproducible assay development, but also raises the safety issue for regenerative medicine. Thus, with the purpose of synchronizing human PSC-derived interneuron cultures to the postmitotic stage, we tested multiple reagents that can modulate cell cycle exit of human interneurons precursors, using Ki67 expression as a proliferating progenitor marker. We analyzed the effect of overexpression of interneuron-specific proneural gene ASCL1 (Mash1), as well as small molecule treatments, such as CultureOne (Life Technology), gamma-secretase inhibitor (DAPT), or fluorodeoxyuridine (FdU) for modulation of interneuron cell cycle exit. CultureOne supplements significantly decreased Ki67+ proliferating progenitors in human interneurons preparation derived from multiple human PSCs, including iPSCs. ASCL1 also showed significant reduction of proliferating progenitors, though with bit milder extent than CultureOne. There was no significant change in proliferating progenitor populations by treatment of DAPT or FdU. While reducing proliferating progenitors, ASCL1 overexpression and CultureOne treatment did not alter the phenotype of interneurons, shown by comparable Sox6- and GAD1-expressing neurons between treated and untreated samples. These results provide a practical and useful way to synchronize human PSC-derived interneurons to generate more reproducible and safe populations of human PSC-derived interneurons for bioassays and cell therapy.

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T-3057

DEDIFFERENTIATED FAT CELLS CONVERT TO PERICYTES PHENOTYPE AND PROMOTE NEOVASCULARIZATION IN MICE

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Although transplantation of mature adipocyte-derived dedifferentiated fat (DFAT) cells into ischemic tissue enhances angiogenesis and increases vascular flow, there is little information about how DFAT cells interact to vascular endothelial cells. We examined the influence of DFAT to vascular endothelial cells on proliferation and tube formation, compared with adipose-derived stem cell (ASC). In the co-culture system, we also examined the differentiation potential of DFAT cells or ASCs into pericytes by immunocytochemistry and realtime RT-PCR. As the results, DFAT cells and ASC coculture promoted proliferation and tube formation of vascular endothelial cells as a similar level. The expression of pericyte markers NG2 and PDGFR-β in DFAT cells was significantly increased by co-culture with vascular endothelial cells. These findings suggest that DFAT cells might convert to pericytes phenotype and promote neovascularization.

T-3059

GENERATION OF HEMATOPOIETIC CELLS FROM MOUSE PLURIPOTENT STEM CELLS IN A 3D CULTURE WITHIN A SELF-ASSEMBLING PEPTIDE HYDROGEL

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Objectives: In vitro generation of HSCs from pluripotent stem cells (PSCs) can be regarded as novel therapeutic approaches for replacing bone marrow (BM) transplantation without immune rejection or graft versus host disease. To date, many differentiation approaches have been evaluated in terms of directing PSCs toward different hematopoietic cell types, yet, low efficiency and no function restrict the further hematopoietic differentiation study, our research aim to develop a three dimention (3D) hematopoietic differentiation approach that serve as recapitulation of embryonic development in vitro to a degree of complexity not achievable in a two dimention (2D) culture system. Methods: Mouse pluripotent stem cells(mPSCs) were induced to hematopoietic differentiation in three dimention self-assembling peptide hydrogel with hematopoietic related cytokines. Flow cytometric analysis detected the hematopoietic makers such as flk1/ckit/CD34/CD41/CD45, Colony-forming cells assay was used to confirm the multipotential hematopoietic differentiation, Six to eight-week-old NOD/SCID mice that were sublethally irradiated were used to evaluate the engraftment potential in vivo. Results: We first found that mouse pluripotent stem cells could be efficiently induced to hematopoietic differentiation with the relative high expression of the hematopoietic related makers such as flk1/ckit/CD34/CD41/CD45. Colony-forming cells assay results suggested mPSCs could differentiated into multipotential hematopoietic stem cells or multipotential progenitors. Animal transplantation experiment showed that mPSCs(CD45.2) could embedded into NOD/SCID mice(CD45.1) with about 10% engraftment efficiency after 3 weeks transplantation. Conclusion: We first developed the 3D inducement approach that could efficiently promoted the hematopoietic differentiation of mPSCs in vitro and obtained the multipotential progenitors that owned the short engraftment potential. Key words: Mouse pluripotent stem cells; Hematopoietic differentiation, Three dimention self-assembling peptide hydrogel ;

T-3061

CHARACTERIZATION OF HIPSC-NEURONS FROM PSYCHOSIS PATIENTS WITH NEUREXIN-1 DELETIONS

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Neurexin-1 (NRXN1) is a highly alternatively spliced presynaptic cell-adhesion protein essential for synaptic function. Heterozygous intragenic deletions in NRXN1 are strongly associated with schizophrenia and autism spectrum disorder. Animal models of NRXN1 deletions and human induced neurons with engineered heterozygous NRXN1 deletions exhibit deficits in synaptic transmission and neurotransmitter release; however, the molecular mechanisms affecting the penetrance of NRXN1 deletions and the functional consequences of

patient specific NRXN1 mutations remain unresolved. Using a rare cohort of human induced pluripotent stem cell (hiPSC) derived neurons from four individuals with heterozygous NRXN1 deletions, we have performed targeted single molecule long read sequencing along with short read sequencing to identify and quantify the complete repertoire of NRXN1α isoforms in this cohort of hiPSC-derived neurons. The short read sequencing results show that NPCs and neurons generated from individuals with NRXN1 deletions display differential NRXN1 expression at the gene, transcript and exon level. Preliminary analysis of long read sequencing from hiPSC neuron of 5 individuals has identified hundreds of unique NRXN1α isoforms, with many differentially expressed isoforms in individuals with NRXN1 deletions. Future work will focus on manipulating these differentially expressed isoforms and understanding their functional significance using a multi-electrode array. Overall, we hope these studies will help to understand how NRXN1 deletions contribute to the genetic risk for neuropsychiatric disorders.

Funding Source: NIH, NYSCF, NARSAD

T-3063

MODELING CHOLESTASIS IN VITRO WITH HUMAN IPSC-DERIVED HEPATOCYTES TO MINIMIZE RISK OF DRUG INDUCED LIVER INJURY

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Drug induced liver injury is most often responsible for the failure of pharmaceutical agents during drug development and cholestasis is the most severe manifestation of drug induced liver disease. Cholestasis is characterized by a mishandling of bile acids in the liver, which leads to both intrahepatic and extrahepatic liver disease. However, the biological mechanisms which trigger cholestasis are not fully understood. Preclinical studies are limited due to their reliance on animal studies, which do not recapitulate human physiology. Development of in vitro model systems descriptive of human liver biology will improve our understanding of drug efficacy and safety. In particular, the application of human iPSCs engineered to carry specific genetic mutations and differentiated towards cell types of the liver will enable us to identify the biological significance of these genes and identify molecular signatures of cholestasis. Early work has focused on optimizing the differentiation of human iPSCs towards hepatocyte like cells. We have demonstrated the functional maturity of these cells through gene expression, flow cytometry and immunohistochemistry. In addition, we have demonstrated their ability to transport cholysyl-fluorescein, a fluorescently labeled bile acid, into the bile canaliculi by BSEP and MRP2 transporters. This functionality may be manipulated in a biologically relevant manner with FXR agonism and highlights the functional maturity of these cells. We have engineered iPSC lines using gene editing to knock out a critical hepatocyte transporter, ABCB4. Following hepatocyte differentiation, these cells demonstrate maintained transporter function, mitochondrial activity and cell viability. Further analysis of the cellular phenotype using RNAseq may help to identify molecular signatures of cholestasis and further classify the significance of this transporter in maintaining hepatocyte biology in vitro. Once we have developed an appropriate in vitro model of cholestasis, we may use it as a benchmark to assess whether drug candidates impact liver biology in vitro as well as determine the significance of novel genes implicated in genome wide association studies for their association with cholestasis.

T-3065

TUNING THE HUMAN DM1 CARDIAC PHENOTYPE THROUGH HIPSC-DERIVED CARDIOMYOCYTES

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Myotonic Dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults, caused by a CTG repeat expansion in DMPK gene and characterized by a variety of multisystemic features. Cardiac involvement is a prevalent phenotypical aspect in DM1 patients and commonly includes defects in conduction system, tachyarrhythmias and dilatative cardiomyopathy. Due to the inaccessibility of heart tissue, we used a patient-specific induced pluripotent stem cell-derived cardiomyocytes (CMs) to recapitulate in vitro DM1 pathogenic mechanism. hiPSCs have been generated from two DM1 patients and two healthy subjects (WT) and differentiated into cardiomyocytes, comparing CMs DM1 respect to WT ones. CMs express structural cardiac markers and intranuclear foci typical of the disease, as well as transcript markers abnormally spliced. Moreover an altered nuclear morphology have been observed in DM1 CMs, due to a dysregulated lamin A/C expression and to prelamin A abnormal accumulation. Electrophysiological analysis specifically evidences in DM1 ventricular-like

CMs an AP profile characterized by an evident delay during depolarising and repolarising phase. The effects of antiarrythmic drug Flecainide perfusion highlight functional electrophysiological abnormalities of DM1 CMs. The quantitative expression of cardiac ion channel genes, involved in CMs electrical behaviour, has revealed a strong down regulation in CMs DM1 compared to WT ones. When analysed by Atomic Force Measurements, CMs DM1 display a diminution of the beating force over time and an unstability of the beating period both in term of frequency and synchronicity, which has never been observed in the CMs WT. These results strongly reproduce human DM1 cardiac phenotype, further enhancing the applicability of hiPSC-CMs for disease modelling and drug discovery. Moreover such findings provide novel insights into the mechanisms leading to the development of arrhythmogenesis and dilatative cardiomyopathy. These aspects have to be taken into account when approaching to DM1 patients, expecially for the risk assessment of sudden cardiac death.

Funding Source: The study was supported by by ASI, "Contromisure per le REAzioni degli astronauti" (COREA) project

T-3067

RESCUE OF FRAGILE X SYNDROME WITH EPIGENOME EDITING TOOLS IN A IPSC-BASED MODEL

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Fragile X syndrome (FXS), the most common form of inherited intellectual disability, is caused by the silence of the fragile X mental retardation 1 (FMR1) gene. Majority of FXS patients carry a CGG expansion in the 5'-UTR of FMR1 resulting in DNA hypermethylation and formation of heterochromatin and subsequently silence of FMR1 expression. FXS iPSCs retain these features at the FMR1 locus and thus represent a unique model to study the mechanism for FMR1 silencing. We previously generated a DNA methylation editing tool consisting of a catalytically inactive Cas9 fused with Tet1/Dnmt3a and target gRNAs that allow for DNA demethylation/methylation in a targeted manner. Our preliminary study showed that targeted demethylation of CGG repeats can reactivate FMR1 in iPSCS and in vitro derived neurons derived from FXS patients. We are further studying the dynamics of FMR1 reactivation by dCas9-Tet1/gRNA and the rescue of FSX phenotypes at the cellular level. Our study provides a proof-of-principle strategy for application of the epigenome editing tools and patient-derived iPSCs to study the pathology of human neurological disorders.

T-3069

ELUCIDATING THE SYNAPTIC DEFECTS IN HEREDITARY SPASTIC PARAPLEGIAS USING IPSC CO-CULTURE MODELS

Mou, Yongchao[1], Dong, Yi[2], Zhang, Su-Chun[2] and Li, Xue-Jun[3]
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Hereditary spastic paraplegias (HSPs) are a heterogeneous group of neurogenetic disorders characterized by axonal degeneration of cortical motor neurons, a group of large projection neurons (PNs). How the connections between cortical PNs and their targets, spinal motor neurons (MNs), are affected in HSPs remain largely unknown. We have generated iPSCs from fibroblasts of HSP patients and showed that these iPSC-derived cortical PNs recapitulate the disease-relevant axonal phenotypes. Here, we seek to determine the synaptic defects in HSP by establishing a co-culture model for SPG3A. We first generated the channel rhodopsin 2 (ChR2)-EYFP expressing iPSC lines (both normal and SPG3A iPSCs) using CRISPR/Cas9-mdeiated homologous recombination. These normal or SPG3A iPSCs were differentiated into cortical PNs (ChR2+), which were then co-cultured with normal or SPG3A spinal MNs derived from regular iPSCs (ChR2-), respectively. After immunostaining, we observed a dramatic increase in the numbers of the Synapsin+/EYFP+/PSD95+ synaptic clusters in the co-cultures comparing to single culture of cortical PNs. Furthermore, the electrophysiological analysis revealed robust evoked postsynaptic currents in spinal MNs after the activation of co-cultured ChR2+ cortical PNs using blue light stimulation, indicating the formation of functional synaptic connections between co-cultured cortical PNs and spinal MNs. Finally, to evaluate the synaptic defects in SPG3A co-culture model, we compared the synaptic connections between cortical PNs and spinal MNs in different groups using both immunostaining and electrophysiological analyses. The number of Synapsin+/EYFP+/PSD95+ synaptic clusters in SPG3A co-culture models was significant reduced comparing to normal co-culture group. The impaired synaptic connections in SPG3A co-culture models were further supported by the dramatic decrease in the frequency of spontaneous excitatory postsynaptic currents (sEPSC) recorded in SPG3A spinal MNs after the activation of ChR2-expressing cortical PNs. Taken together, our data reveal the impaired synaptic connections between cortical PNs and spinal MNs in a SPG3A co-culture model, which will serve as a unique system to study the

pathogenic mechanism and explore the treatment for HSPs.

Funding Source: This work has been supported by the Blazer Foundation and the NIH (R21NS089042).

T-3071

EARLY iPSC REPROGRAMMING AND MURINE DEVELOPMENT BOTH REQUIRE ACTIVE DNA DEMETHYLATION

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Over the last sixty years, seminal studies have demonstrated that differentiated cells can be reprogrammed to pluripotency by somatic cell nuclear transfer,cellfusion,andtheoverexpressionoftranscription factors. However, whether the three approaches share a common mechanism of reprogramming is still unclear. Previous work from our laboratory demonstrated that active DNA demethylation by the deaminase AID is required for cell fusion initiated nuclear reprogramming. While heterokaryons are amenable to the study of active DNA demethylation due to their inherent lack of DNA replication and cell division, here we test whether a conserved active DNA demethylation mechanism is also required in the formation of rapidly dividing induced pluripotent stem cells (iPSCs). To this end, we implement a novel approach to investigate the mechanism of active DNA demethylation in iPSCs where cell division and DNA replication are ongoing and essential. Previously, we, and others, show that AID KO MEFs reprogram normally, but by contrast, acute early AID knockdown by RNA interference (RNAi) blocks reprogramming suggesting compensation by an unknown factor upon genetic loss of AID. Here, we show that the hydroxylase TET1, and not TET2, compensates during the constituent genetic loss of AID in murine embryonic fibroblasts (MEFs) and observe a de novo methylation dependent increase in TET1 mediated demethylation after acute AID loss. Additionally, by increasing the activities of both AID and TET1 early in reprogramming by cytokine and chemical stimulation, colony formation occurs in half the time (4-days) and the efficiency of iPSC reprogramming is dramatically increased (~20-fold). Furthermore we report that AID and TET1 are both required for continuous methylation fidelity and active demethylation of DNA during both iPSC formation and murine development and herein reveal a key role for AID in balancing the opposing forces of methylation.

Funding Source: Life Science Research Foundation, Howard Hughes Medical Institute, Baxter

T-3073

SINGLE CELL SIGNALING DYNAMICS DURING SOMATIC CELL REPROGRAMMING

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Generation of induced Pluripotent Stem Cells (iPSCs) from somatic cells is a revolutionary yet extremely inefficient process. Ectopic expression of Oct4, Sox2, Klf4 and c-Myc (OSKM) in somatic cells leads to dramatic changes in gene expression and chromatin remodeling that, in less than 1% of cells, culminate with the formation of a colony of pluripotent cells. Even in isogenic cell populations synchronously expressing the OSKM cassette, single cells exhibit a vast heterogeneity at many levels including cell morphology, cell cycle, and gene expression. Understanding the key molecular characteristics and dynamics of successfully reprogrammed cells would greatly facilitate progress in regenerative medicine. One of the most physiologically relevant changes occurring in this transition involves rewiring of signaling networks. Using live cell imaging and signaling biosensors we have measured signaling network dynamics at single cell resolution and thus captured rewiring of somatic cells during reprogramming. Using these tools, we have observed altered frequency of ERK activity oscillations in mouse embryonic fibroblasts (MEFs) undergoing reprogramming. By interrogating these network dynamics with growth factor stimulations, we have seen that as MEFs accelerate their cell cycle and lose fibroblast morphology they also exhibit different signaling responses compared to primary MEFs. We are utilizing existing biosensors and engineering novel reporters to expand these observations to other signaling pathways with the aim of investigating how signaling heterogeneity contributes to success or failure of reprogramming in single cells.

Funding Source: National Science Foundation Graduate Research Fellowship Program

T-3075

BRIEF EXPOSURE TO SMALL MOLECULAR COMPOUNDS ALLOWS INDUCTION OF MOUSE EMBRYONIC FIBROBLASTS INTO NEURAL CREST-LIKE PRECURSORS

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Gene-transfer techniques are a common, efficient method for cell fate conversion, including generation of induced pluripotent stem cells. Additionally, recent studies have suggested that small molecules can alter signal transduction pathways, cell morphology, and cell fate. In this study, we propose a novel method for inducing non-neuronal cells into peripheral neurons via neural precursors without the use of gene-transfer methods. Cells were briefly exposed to small molecule cocktails containing epigenetic modulators and specific signal inhibitors in a step-by-step manner, followed by neuronal differentiation. Immunochemical staining and calcium flux assays revealed the generation of mature peripheral neurons from mouse embryonic fibroblasts and adult somatic cells. In addition, time-lapse imaging of neural precursor-specific enhancer expression, quantitative polymerase chain reaction assays, and global gene expression analyses showed that neurons were generated by passing thorough a neural crest-like precursor stage. Consistent with these results, the chemically induced mouse neural crest-like cells differentiated into sympathetic neurons, adipocytes, osteocytes, and smooth muscle cells, which are characteristic lineages of neural crest-derived cells. Therefore, these results indicated that brief exposure to chemical compounds could induce a multipotent cell without viral transduction. Our induction method is a promising approach that may contribute to the development of therapeutic applications as a regenerative medicine.

T-3077

ENGINEERED PLATFORMS FOR NEURAL STEM AND PROGENITOR CELL RESEARCH

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Oligodendrocyte progenitor cells (OPCs) are a class of multipotent stem-like cells that, when differentiated properly, engage and enclose neuronal axons with a myelin sheath. Poor remyelination, due to hindered OPC migration, axon engagement, or differentiation, is associated with poor nervous system function in diseases such as multiple sclerosis. Understanding causes and potential treatments of disorders characterized by incomplete myelin production or myelin degeneration are particularly challenging due to a lack of preclinical, in vitro tools that replicate key aspects of the OPC-neuron interactions. Emerging research including our own suggests that mechanosensitivity of the oligodendrocyte lineage, and physical and mechanical characteristics of axons, may impact key features of myelination such as the onset of oligodendrocyte differentiation, thickness and length of the myelin segments, and the structure of nodes of Ranvier. Here we discuss the development of engineered, three dimensional arrays of polymeric fibers that serve as mimetics of neuronal axons, using a combination of materials engineering and high resolution 3D microfabrication, which enable study of OPC engagement and subsequent myelination in vitro. Using conventional microscopy techniques and high-throughput analysis methods, we show cell-material interactions in these artificial axons are maintained. These tools now facilitate fundamental studies of how these stem and progenitor cells respond to specific geometric, mechanical, and chemical cues in the complex environment of the central nervous system.

T-3079

MULTILAYER NANOFILM FOR STABILIZING GROWTH FACTOR AND MAINTAINING UNDIFFERENTIATED STATE OF HUMAN INDUCED PLURIPOTENT STEM CELLS

Park, Ju Hyun[1], Choi, Moonhyun[2], Park, Hee Ho[3], Han, Uiyoung[2], Kim, Yu Jin[4] and Hong, Jinkee[2]
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Human pluripotent stem cells (iPSC) are dependent on basic fibroblast growth factor (bFGF) in their growth medium to maintain their undifferentiated state. The instability of bFGF means that it must be frequently added to culture medium, yet loss of bFGF activity can still occur. To protect against denaturation, bFGF should be preserved under mild conditions and inside novel containers such as microspheres, hydrogels, or nanofilms. In the least amount of time, fabricating nanofilm and handling sensitive materials without detriment to activity via highly productive manufacturing technology are significant for practical uses in the field of biomedical applications. Herein, a layer-by-layer technique-based nanofilm system consisting of charged polymeric materials was established for the sustained release of bFGF. The bFGF-releasing nanofilm was constructed and human iPS cells were cultured with the nanofilm. As a result, human iPS cells maintained their undifferentiated morphology and expression of pluripotency markers including SSEA-4, Oct4 and alkaline phosphatase even with less frequent media changes. Furthermore, we developed a multilayered nanofilm fabricating system by inkjet printing to incorporate bFGF successfully. We demonstrated that water mixed with glycerol as biological ink maintained stability of bFGFs through simulation and experimental study. With highly stable bFGFs, we were able to enhance the proliferation of human dermal fibroblast (HDF) and maintain the undifferentiated state of induced pluripotent stem (iPS) cell by the controlled release of bFGF. It is anticipated that the controlled release of bFGF from the biocompatible nanofilm reduces the frequency of media replacement needed to maintain stem cell cultures.

Funding Source: This research was supported by the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT and Future Planning of the Korean government 2016M3A9C6917402 and 2015R1C1A1A01052831.

T-3081

ISOLATION AND IDENTIFICATION OF MESENCHYMAL STEM CELL FROM PAP-SMEAR TEST

Park, Junghyun and You, Seungkwon

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Stem cells are generally defined as clonogenic cells capable of both self-renewal and multilineage differentiation. For this reason, stem cells are promising sources for the regenerative medicine of tissue and organ systems. Mesenchymal stem cells (MSCs) are widely used in clinical medicine because of the differentiation potential into lineages of mesenchymal tissues (cartilage, bone, fat, muscle) and the comprehensive paracrine system. Adult MSCs can be isolated from many types of tissue such as bone-marrow, adipose, synovium, and amniotic fluid. In this study, new and simple method for isolation of MSCs was discovered. Papanicolaou test (PAP-smear test) is a method of cervical screening used to detect potentially pre-cancerous and cancerous processes in the cervix and diagnose the genetic diseases of fetus. When PAP-smear test conducted, many numbers and types of cells are collected. We attempted to establish the cell line which can be cultured in vitro from the PAP-smear test samples. Acquired cell line showed the mesenchyme lineage-specific characteristics, high proliferation rate, and multipotent differentiation (adipocyte, osteocyte, chondrocyte). Overall, mesenchymal cells from the PAP-smear test is the novel source of mesenchymal stem cells for clinical approach.

Funding Source: This work was supported by a grant of the Korea Health Technology R&D Project, funded by the Ministry of Health & Welfare (HI15C0810) and School of Life Sciences and Biotechnology for BK21 PLUS, Korea University.

T-3083

USING NETWORK BIOLOGY TO DERIVE A QUANTITATIVE BASIS FOR DEFINING CELL IDENTITY WITH APPLICATIONS TO SINGLE CELL RNA-SEQ AND CELL FATE ENGINEERING

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Cell fate engineering, for example the directed differentiation of pluripotent stem cells (PSC) or the direct conversion among somatic cell types, is practiced in thousands of labs worldwide to model diseases, to explore inaccessible time points in development, to screen drugs, and to develop regenerative medicine

therapies. However, one daunting barrier that impedes cell fate engineering from fulfilling its promise to broadly transform the biomedical enterprise is the lack of generally applicable tools to comprehensively assess the identity (or fidelity) of engineered cells. Gene regulatory networks (GRNs) are programs encoded in the genome that define the set of regulatory relationships among genes and gene products. GRNs govern the cell's transcriptional outputs both at the steady state and in response to perturbations, and thus are major molecular determinants of cell-type identity. We previously developed a computational platform called CellNet that uses GRNs to measure the extent to which engineered cells are equivalent to the intended target cell type. Here, we describe our extension of CellNet so that it achieves a cell type level of granularity by applying to single cell RNA-Seq (scRNA-Seq) from either human or mouse. Two prerequisites to this were (1) the creation of a standardized pre-processing pipeline for a variety of scRNA-Seq methods, and (2) the development of a novel, GRN-based method to define cell type de novo in an unsupervised fashion. We illustrate how our tool can be applied to scRNA-Seq data from cell fate engineering experiments to not only determine the cell type composition of engineered populations, but to also quantify the quality of each cell relative to the intended target cell types.

T-3085

DEVELOPMENT OF A VERSATILE MEDIA SYSTEM FOR CARDIOMYOCYTE DIFFERENTIATION IN 2D- OR 3D- CULTURE SYSTEMS USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) can be used in various applications including regenerative medicine, drug discovery and safety assessment. Further, hiPSC-CMs can be generated through three-dimensional (3D) embryoid body formation or two-dimensional (2D) monolayer methods. Although 3D embryoid protocols generate larger hiPSC-CM yields, use of costly growth factor supplements (Activin A, BMP4, Dkk-1, VEGF etc.) are necessary, which can cause variations in differentiation efficiency according to inconsistencies in the supplements used. The 2D method is made possible through the use of chemical compounds (CHIR99021, IWP2 etc.), but generates hiPSC-CMs typically at low yields. Both 2D and 3D methods feature positive and negative aspects, and may present further complications where hiPSC lines may demonstrate differential yields in 2D and 3D systems. This may involve additional cost and labor to determine optimal conditions for maximal hiPSC-CM yield, which may be specific only to 2D or 3D methods. We have developed a media formulation that can be applied to both 2D and 3D culture systems using chemical compounds, resulting in a versatile, cost-effective hiPSC-CMs production system. Here, we characterize our media system compared to conventional methods in various iPSC lines using immunocytochemistry and flow cytometry.

Funding Source: This work was supported by Kyoto Economic Gardening Support Fund, by KYOTO Industrial Support Organization 21

LATE BREAKING POSTER SESSION II-EVEN 19:00 – 20:00

T-3004

MICRORNA-210 IMPROVES THE SURVIVAL POTENTIAL OF ADULT MOUSE ATRIAL PROGENITORS

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Although stem cell therapy has provided a promising treatment for myocardial infarction (MI), the low survival of the transplanted cells in the infarcted myocardium is a possible limitation to long-term improvement. Therefore, the development of strategies to enhance stem cell survival is of importance to this field. We have previously shown that miRNA-210-3p protects mouse atrial progenitors from serum starvation-induced cell death by reducing caspase gene expression and activity. In this study, we further examine the effects of miRNA-210-3p on serum-starved mouse atrial progenitors. Adult mouse atrial progenitors (n=3) were expanded as slowly adhering collagenase-trypsin cells (CTs), a heterogeneous population that expresses the cardiac progenitor and developmental markers Nkx2.5, GATA4, MEF2c, cKit and Sca1. CT survival was assessed by serum starvation following transfection with miRNA-210-3p using DharmaFECT. Serum starvation induced cell death, with surviving cells having decreased expression of the cardiac progenitor marker Sca1 and increased expression of the cardiomyocyte marker Troponin T. MiRNA-210-3p partially reversed this effect. The cardiomyocyte differentiation potential of miRNA-210-3p-transfected CTs was preserved following differentiation using the Goumans protocol. In addition, miRNA-210-3p improved the paracrine function of CTs by inducing IGF-1 secretion, which is involved

in neovascularisation and cardiac repair. Finally, the consequences of miRNA-210-3p overexpression on cellular functions such as autophagy were investigated. Analysis with TargetScan and Microcosm bioinformatics tools revealed miRNA-210-3p potential target sequences in the 3'UTR regions of two essential autophagy genes: ATG4c and ATG7. Therefore, expression of these ATGs as potential targets was assessed. Due to the relocalisation of LC3-II to autophagosomal membranes during autophagy, LC3-II puncta were quantified to indirectly measure autophagic flux. However, there was no significant change in the expression levels of the ATGs or numbers of LC3-II puncta in serum-starved CTs. In conclusion, miRNA-210-3p enhanced the survival and paracrine secretion of cardiac progenitors without adversely affecting differentiation potential.

Funding Source: King Faisal Specialist Hospital & Research Centre

T-3006

SINGLE-CELL ANALYSIS IDENTIFIES DISTINCTLY DIFFERENT POPUIATIONS IN CIUCULATING STEM AND PROGENITOR CELLS IN PERIPHERAL BLOOD WIHTOUT MOBILIZATION

Yulin, Xu, Li, Xia, Wang, Bensheng, Shan, Wei, Chen, Haide, liu, Senquan,Tie, Ruxiu, Long, Yan, Cai, Shuyang, Zhang, Hao, Li, Honghu, Qiu, Yunfei, Xu, Huijun, Yu, Xiaohong and Huang, He
Zhejiang University, Hangzhou, China

Rare numbers of stem and progenitor cells are circulating in human peripheral blood (PB) without mobilization. To study the identities of the circulating stem and progenitor cells (cSPCs), we coupled three dimension cultures with single-cell transcriptome sequence of human peripheral blood monocytes without mobilization. The results showed that types of populations included distinct morphology with compact colonies (3D-CC), loose cobble colonies (3D-LC), and free cells (2D) presenting in the three dimension cultures. Principal component analysis (PCA) analysis based on differential expression genes divided the culture populations into subgroups. The transcriptional sequence analysis further demonstrated the hierarchy hematopoietic signatures among the subgroups. At the interface of 3D-CC and 2D signatures, some hematopoietic stem cell signature and endothelial cell relevant properties were displayed. Multi-potential capability of cSPCs was verified via colony-forming unit and reconstitute transplantation assays in vitro and in vivo. The findings demonstrated a more real case for the rare stem and progenitor cells circulating in peripheral blood under normal physiological condition.

Funding Source: The research was supported by National key basic research program (grant # 2015CB964900), and International Cooperation and Development Fund (grant # 81520108002). Funding sponsors had not taken part in the study.

T-3008

LIVER BUD ORGANOIDS AS AN IN VITRO MODEL FOR STUDYING THE ROLE OF IPSC-DERIVED HUMAN FETAL LIVER CELLS IN THE HEMAPOIETIC MAINTENANCE AND PROGENITOR EXPANSION

Philonenko, Elena S.[1], You, Hao[1], Libedin, Mikhail[2], Kiselev, Sergey[2], Lagarkova, Maria[2] and Samokhvalov, Igor[1]

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Mammalian fetal liver supports active hematopoiesis and maintains hematopoietic stem and progenitor cells starting from midgestation and beyond birth. The hepatic endoderm compartment of fetal liver is thought to play a key role in the hematopoietic progenitor maintenance and/or expansion. We have developed a novel and highly efficient protocol for human PSCs differentiation into fetal liver cells. For functional characterization of the hPSC-derived hepatic cells we have employed the fetal liver bud self-assembly (Takebe et al., Nature 2013) for testing their ability to maintain and/or expand human hematopoietic progenitors. We used self-assembled artificial liver bud organoids as in vitro model for studying the role of different fetal liver cell types in the hematopoietic maintenance and progenitor expansion. We have adopted the original liver organoid self-assembly protocol to well-defined cell culture conditions and for maintenance and proliferation of early blood progenitors derived from human PSCs. To investigate hematopoiesis-supporting role of the fetal liver organoids, we introduced CD43posCD45neg early human hematopoietic progenitor cells into the liver bud cell mix containing hMSCs, HUVECs and hepatic endoderm cells. AFPpos/HNF4alphapos hepatic endoderm cells were generated from hPSCs according to our optimized protocol. CD43posCD45neg cells within the artificial tri-lineage liver bud environment were able to develop into CD43negCD4pos cells that showed clear signs of terminal differentiation. After one-week liver bud culture practically all CD43negCD45pos cells relocated into the internal region of the organoid made up of CD105pos hMSCs/HUVECs. In contrast, all cells that preserved their progenitor CD43posCD45neg phenotype remained in close association with CK8pos or AFPpos hepatocytes localized mainly in the external region of the liver buds. These observations suggest

that the endothelial/mesenchymal compartment of artificial liver bud permits overt differentiation of early human hematopoietic cells, whereas close contact of the blood progenitors with hepatocyte compartment helps to maintain their undifferentiated state. Thus, our novel protocol of hPSC hepatocyte differentiation generates hepatic endoderm cells with a specific functional signature.

Funding Source: The research was founded by RFBR grant 15-04-05675 A

T-3010

GENERATION OF LONG-TERM REPOPULATING HEMATOPOIETIC STEM CELLS FROM NON-HEMOGENIC ENDOTHELIUM

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In vitro production of hematopoietic stem cells (HSCs) from pluripotent stem cells remains a challenge. Unlocking the roles of accessory cells at sites of HSC production may help clarify whether these cells or their products constitute a missing element in current protocols. HSCs originate from hemogenic-endothelium that lines the ventral surface of the dorsal aorta in E10.5 embryos. We have identified distinct PDGFRA+/Nestin-GFP+ (N-GFP) and PDGFRA+/N-GFP- cells populations with colony forming unit-fibroblast (CFU-F) activity in the E11.5 AGM. Whereas PDGFRA+/N-GFP- CFU-Fs in the AGM can be serially passaged and show long-term clonogenicity, PDGFRA+/N-GFP+ CFU-Fs show limited self-renewal. These AGM CFU-Fs showed in vitro differentiation potential into mesodermal and ectodermal derivatives but those of the endoderm. PDGFRA-/N-GFP+/PDGFRB+/CD31+ cells possess haematopoietic CFU-C potential whereas PDGFRA+/N-GFP+/PDGFRB+/CD31- cells lack CFU-C but retain CFU-F potential. Conditional ablation of PDGFRA+/Nestin+ or PDGFRA+/Nestin- cells in the AGM led to either partial or complete loss of CFU-Fs respectively, with severe loss of endothelial and pericyte-like cells and concomitant loss of blood formation. PDGFRA+ cells progressively acquire Nestin expression as they migrate towards the aortic lumen. Tamoxifen induced lineage tracing in PDGFRACreERT2/R26eYFP mice showed that stromal, sub-endothelial, endothelial and hematopoietic cells including long term repopulating HSCs (LT-HSCs) in E11.5 AGM are progeny of PDGFRA cells. To trace the origins of these stromal cells, we used Mesp1/R26eYFP (mesoderm) and Wnt1/R26eYFP (neural crest) reporter mice and observed that MesP1 derived PDGFRA+ cells dominated the sub-endothelial and deeper ventral stroma in the AGM at E11.5 but are replaced by Wnt1 derived cells by E13.5. In vitro co-aggregation of E11.5 Mesp1 derived PDGFRA+/PDGFRB-/CD45-/CD31-/VE-CAD- MSC-LC cells with E13.5 or adult cardiac non-hemogenic endothelial cells (PDGFRA-/PDGFRB-/CD45-/CD31+/VE-CAD+) resulted in the generation of endothelial cell derived LT-HSCs, which could be abrogated by dose dependent inhibition of PDGFRA signalling.

Funding Source: NHMRC New-Investigator Project Grant

T-3014

DIFFERENTIAL GROWTH TRIGGERS MECHANICAL FEEDBACK THAT ELEVATES HIPPO SIGNALING

Pan, Yuanwang[1], Heemskerk, Idse[2], Ibar, Consuelo[3], Shraiman, Boris[2] and Irvine, Kenneth[3]
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Mechanical forces have emerged to be an important regulator of tissue growth. For example, high cytoskeletal tension enhances tissue growth while low cytoskeletal tension decreases tissue growth. On the other hand, growth has also been suggested to affect tissue mechanics: heterogeneous growth could lead to mechanical stress that feedbacks into cells to maintain growth homeostasis. However, whether and how such a mechanical feedback mechanism functions in vivo are not clear. Here we test the mechanical feedback hypothesis by inducing differential growth in Drosophila wing disc epithelia through distinct approaches. We show that differential growth triggers a mechanical response that lowers cytoskeletal tension along apical cell junctions within faster-growing cells. This reduced tension modulates a biomechanical Hippo pathway, decreasing recruitment of Ajuba LIM protein and the Hippo pathway kinase Warts to junctions, thus reducing the activity of the growth-promoting transcription factor Yorkie. This provides the experimental support and a molecular mechanism for lowering growth rates within faster-growing cells by mechanical feedback. Additionally, bypassing mechanical feedback induces

tissue distortions and inhomogeneous growth. Thus our research further identifies the roles of mechanical feedback in maintaining tissue shape and controlling patterned growth rates during development.

T-3016

BIOMECHANICS OF THE MURINE BONE MARROW NICHE IN HEMATOPOIETIC STEM AND PROGENITOR CELL FATE DECISIONS

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Recent studies suggest that the biomechanical properties, including extracellular matrix (ECM) and elasticity, of the bone marrow (BM) niche microenvironment may affect hematopoietic stem cell (HSC) function. We are interested in comparing the long-lived, self-renewing HSCs with the short-lived, myeloid-lineage committed granulocyte macrophage progenitor (GMP), to determine how distinct hematopoietic populations with different fate potential integrate biophysical cues from their niche. At steady state, HSCs preferentially localize the bone endosteum (END) and perivasculature (PV), while GMPs are found scattered throughout the BM cavity, including the central marrow. In vitro adhesion analyses indicate that HSCs preferentially bind to fibronectin, the most abundant ECM in the central marrow (CM). GMPs adhere to collagen I, an ECM found at the END, where GMPs preferentially expand during myeloid regeneration. In vitro adhesion assays and scanning electron microscopy analyses reveals that quiescent HSCs are less adherent than activated HSCs and may have different glycocalyx layer formation. Using atomic force microscopy (AFM) as well as various immunofluorescence staining approaches, we created a biomechanical map of the elastic modulus and ECM composition of the END, PV and CM, which we also modeled in vitro using 2D polyacrylamide gels (PAGs). We observed that HSC proliferation is correlated to environmental elasticity, while GMP proliferation is inversely correlated. Moreover, HSCs cultured on an END-like PAG showed greater in vitro colony formation in methylcellulose and better long-term multilineage engraftment upon transplantation than HSCs cultured on CM- or PV-like PAGs. In disease conditions, the BM can become fibrotic, changing the ECM composition and the elasticity of BM niches. This may contribute to the clonal dominance of transformed HSCs. We have shown that transformed HSCs have better adherence to fibrotic collagens than healthy HSCs. We are currently modeling the effect of fibrosis on BM elasticity using AFM, and are probing this effect on HSC function using PAGs. Our results provide the first characterization of the physical features of distinct BM niche microenvironments, and their role in controlling HSC and progenitor fate decisions in normal and disease conditions.

T-3018

PROTECTIVE EFFECTS OF OPHTHALMIC SUPPLEMENTS IN THREE-DIMENSIONAL RETINAL ORGANOIDS DIFFERENTIATED FROM MOUSE IPS CELLS

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We have previously reported that we induced photoreceptor cell death in mouse iPS cell-derived three-dimensional retinal organoids (3D-retinas), and established a live-cell imaging system to measure disease-related properties (ISSCR 2015 Annual Meeting). The purpose of the present study is to estimate the protective effects of representative ophthalmic supplements for treating the photoreceptor degeneration and analyzed the mechanism. We generated 3D-retinas from iPS cells established from Nrl-GFP mice that express GFP in differentiated rod photoreceptors. The photoreceptor cell death was induced by 4-hydroxytamoxifen (4-OHT), an inverse agonist for estrogen related receptor β (ERRβ) that is enriched in differentiated rod photoreceptors. We treated 3D-retinas with 4-OHT and examined the protective effects by cotreatment with traditional ophthalmic supplements; vitamin E, lutein, astaxanthin, and anthocyanidin. The time course of GFP fluorescence was measured using a live-cell imaging device (IncuCyte Zoom) to quantify the degeneration and protective effects. 4-OHT-induced photoreceptor degeneration was correlated with attenuation of GFP fluorescence by the immunohistochemistry of retinal sections. In contrast, adding ophthalmic supplements of 4-OHT-treated 3D-retinas resulted in delayed attenuation of GFP fluorescence compared with unsupplemented 3D-retinas in a dose-dependent manner. We also performed microarray analysis of gene expression to understand the mechanism. Microarray data showed that endoplasmic reticulum and oxidative stress-related genes were found to be significantly upregulated in the 4-OHT-treated 3D-retinas compared with vehicle-treated retinas. In contrast, some supplements partially suppressed such upregulation. In conclusion, we succeeded in reproducing in vitro photoreceptor degeneration in 3D-retinas differentiated from mouse iPS cells and examined the protective effects of representative ophthalmic supplements. This drug evaluation system enables us to monitor drug effects in photoreceptor cells and could be useful for drug screening.

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T-3020

YAP IS REQUIRED FOR THE SELF-RENEWAL OF BASAL PROGENITORS IN THE DEVELOPING GYRENCEPHALIC FERRET AND HUMAN NEOCORTEX

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The expansion of the neocortex during mammalian evolution has been linked to an enlargement of the subventricular zone during cortical development and an increase in the proliferation of the basal progenitors (BPs) residing therein. Here, we explored a potential role of YAP, the major downstream effector of the Hippo signaling pathway, in BP proliferation. We show that YAP expression and activity are high in ferret and human BPs, which are known to exhibit high proliferative capacity, but low in mouse BPs, which lack such capacity. To induce YAP activity in mouse BPs, we expressed a constitutively active YAP (CA-YAP). This resulted in an increase in BP proliferation. In addition, CA-YAP–expressing mouse BPs increased the production of upper-layer neurons as compared to control. To investigate if YAP is required for the proliferation of BPs, we pharmacologically interfered with the function of YAP in the developing ferret neocortex. This resulted in a decrease in cycling BPs. Our data indicate that YAP promotes the proliferation of BPs and suggest that changes in YAP activity levels may have contributed to the evolutionary expansion of the neocortex.

T-3024

ENHANCED NEURAL STEM CELL DIFFERENTIATION TOWARD OLIGODENDROCYTES: A NOVEL MECHANISM OF FINGOLIMOD TREATMENT FOR NEURAL REPAIR

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Enhanced neural stem cell differentiation toward oligodendrocytes: A novel mechanism of fingolimod treatment for neural repair Yuan Zhang, Xing Li, Bogoljub Ciric, Abdolmohamad Rostami, and Guang-Xian Zhang Department of Neurology, Thomas Jefferson University, Philadelphia, PA, 19107 Neural stem cell (NSC) differentiation into oligodendrocytes, the myelinating cells, is crucial for neural repair for multiple sclerosis, an inflammatory demyelinating disease of the central nervous system. Fingolimod, the first FDA-approved oral medication for multiple sclerosis, suppresses acute disease but is less effective at the chronic stage, and whether it has a direct effect on neuroregeneration in multiple sclerosis remains unclear. Here we show that fingolimod, at nanomolar concentrations, effectively protected NSC survival and enhanced their development into mature oligodendrocytes in vitro, primarily through the S1P3 and S1P5 receptors. In vivo, treatment with either fingolimod or NSCs alone had no effect on the secondary progressive stage of remitting-relapsing experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. However, a combination therapy with fingolimod and NSCs promoted significant recovery, including ameliorated clinical signs and central nervous system inflammatory demyelination, enhanced myelin basic protein synthesis and remyelination, inhibited axonal degeneration, and reduced astrogliosis. Moreover, fingolimod significantly improved incorporation and survival of transplanted NSCs in the central nervous system, and drove their differentiation into more oligodendrocytes but fewer astrocytes, thus promoting remyelination and central nervous system repair processes in situ. Our data demonstrate a novel effect of fingolimod in NSC differentiation and remyelination, broadening its possible application to NSC-based therapy in the secondary progressive stage of multiple sclerosis.

Funding Source: This study was supported by Novartis Pharma (Basel, Switzlerland) and the National Institutes of Health.

T-3026

ETHANOL UP-REGULATES BACE1 VIA ENDOPLASMIC RETICULUM STRESS INDUCED COX-2 MEDIATED PGE2 SIGNALING THROUGH PKA/CREB PATHWAY

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Alzheimer's disease (AD) is caused by toxic peptide amyloid-β (Aβ) that produced from cleaving of amyloid precursor protein (APP) by beta-site APP-cleaving enzyme 1 (BACE1) and gamma secretase. Previous studies have linked alcohol (i.e., ethanol) adverse effect to AD by different mechanisms. Using human-derived neuroblastoma cell line SK-N-MC, we investigated the effect of ethanol on BACE1 upregulation and Aβ overproduction. Our western blotting and immunostaining results demonstrated that BACE1 was up-regulated by ethanol in a dose-dependent manner.

Ethanol-stimulated ROS led to induction of endoplasmic reticulum (ER) stress markers, Chop expression, and eIF2α phosphorylation. PBA (ER stress inhibitor) inhibited ethanol-increased cyclooxygenase-2 (COX-2) expression and PGE2 production. Using salubrinal (inhibitor of eIF2α dephosphorylation) and EIF2α siRNA, our results indicated that eIF2α phosphorylation mediated ethanol-induced COX-2 expression. COX-2 induced BACE1 upregulation in a PGE2-dependent manner which was abolished by NS-398 (selective COX-2 inhibitor). Moreover, ethanol promoted PKA activation and CREB phosphorylation and translocated them into the nucleus were reversed by PF-04418948 (EP-2 receptor blocker). 14-22 amide (PKA inhibitor) pretreatment and CREB siRNA transfection suppressed ethanol-elevated BACE1 levels. Furthermore, PF-04418948 ameliorated Ethanol-stimulated Aβ secretion. In conclusion, ethanol-induced COX-2 through eIF2α phosphorylation led to BACE1 upregulation via EP-2 receptors engaged PKA /CREB pathway.

T-3028

MODELING ANESTHETIC-INDUCED DEVELOPMENTAL NEUROTOXICITY USING STEM CELL-DERIVED HUMAN CEREBRAL ORGANOIDS: APOPTOSIS, AUTOPHAGY, AND ALTERED MRNA PROFILE

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Studies in young animals and children suggest that general anesthetics early in life have negative effects on the developing brain (e.g., memory and learning disabilities), but mechanisms are largely unknown partially due to the lack of an appropriate human model. The use of induced pluripotent stem cells (iPSCs) to generate 3 dimensional cerebral organoids, has been further developed in our laboratory, in order to utilize a more clinically relevant human model to study the neurotoxic effect of the intravenous anesthetic propofol. Cerebral organoids were generated from human iPSCs by a sequential culture process in chemically defined medium, and characterized by immunostaining of markers specific to neural lineages and patch clamp analysis of electrophysiological properties. Two-month-old cerebral organoids were exposed to a clinically relevant dose of propofol for 6 hours or the dimethyl sulfoxide vehicle control. Cell apoptosis and autophagy were assessed by Western blot and electron microscopy. The effect of propofol on the mRNA expression profile of 18,675 genes was analyzed by Arraystar array, and further confirmed by real time PCR. Two-month-old cerebral organoids include 80% neurons, and 20% neural stem cells and neuron supporting cells (e.g., astrocytes and oligodendrocytes). Neurons formed organized synapses, and displayed functional glutamatergic and gamma-Aminobutyric acid-ergic currents. Propofol for 6 hours increased cleaved caspase 3 expression as an indicator of neuroapoptosis. Electron microscopy revealed that propofol increased autophagy and elicited abnormal mitochondrial morphology. Microarray analysis revealed differential expression of 113 mRNAs, with 49 predicted by bioinformatics analysis to be involved in autophagy, mitochondrial stress, and neurodegeneration. Collectively, these data demonstrate that propofol induces neurotoxicity in cerebral organoids. The alterations in mRNA profile, and abnormal changes in autophagic and mitochondrial processes may contribute to downstream apoptosis. These clinically relevant iPSC-derived human cerebral organoids will provide new insights into neurodevelopmental consequences of propofol exposure, and allow identification of more rational neuroprotective strategies related to pediatric anesthetic use.

T-3030

MZF1 and GABP Cooperate with Sox2 in regulating the expression of YAP1 in Cancer stem cells

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The Hippo pathway discovered in Drosophila as a regulator of organ size, consists of a phosphorylation cascade that restrains the activity of the transcriptional coactivators, YAP1 and TAZ. Hippo signaling in mammals restrains cell proliferation and stemness. This pathway also has a significant tumor suppressive role, as it promotes the phosphorylation and nuclear exclusion of YAP1, which can function as a potent oncogene. We have shown that the stem cell transcription factor SOX2 antagonizes the Hippo pathway and affects the lineage differentiation fates of mesenchymal stem cells (MSCs) through direct induction of YAP1 (Seo et al, Cell Reports, 2013). SOX2 and YAP1 also maintain cancer stem cells in osteosarcomas, a cancer that arises from the MSC lineage. SOX2 antagonizes the Hippo pathway by directly inducing YAP1 and repressing Hippo upstream activators (BasuRoy et al, Nature Comm, 2015). We are now determining how SOX2 and other transcription factors regulate YAP1 expression in the osteogenic lineage by mutagenesis and proteomic analysis. In particular, we are using nucleotide substitutions to delete putative functional elements in the 243 bp enhancer region of YAP1. We have identified additional transcription factor (TFs) Myeloid Zinc Finger 1 (MZF1) and GA Binding Protein (GABP) that play a role in regulation of YAP1 in cancer stem cells. Our data reveal

that Myeloid Zinc Finger domain (MZF1) and GA binding protein (GABP) are required to maintain basal activity of YAP1 in osteoproginator cells. Knocking down of MZF1 and GABP decreases the expression of YAP1 while, overexpression of MZF1 activates YAP1 expression. Interestingly, these and other related studies have led to the conclusion that YAP also regulates SOX2 expression creating self sustaining expression loop. Elevated YAP1 activity due to mutations in Hippo pathway components or YAP1 amplification is observed in several types of human cancers such as osteosarcoma and glioblastomas which also express high level of SOX2. Therefore disruption of YAP1 transcriptional activity could be a therapeutic strategy for YAP1 dependent tumours.

T-3032

X CHROMOSOME INACTIVATION STATUS OF KLINEFELTER SYNDROME DERIVED HUMAN INDUCED PLURIPOTENT STEM CELLS

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X chromosome inactivation (XCI) is a mechanism in which one of the two X chromosomes in female somatic cells is transcriptionally silenced to equalize the dosage of X-linked genes between males and females. Interestingly, XCI occurs as well in male cells of Klinefelter syndrome (KS) patients, characterized by a 47 XXY karyotype. However, the XCI in KS patients is incomplete with a few genes remaining active in the inactivated X chromosome (Xi), contributing to some female phenotypic features and germ cell dysfunctions in KS patients. XCI status in female human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC) lines is variable; with no XCI (Xa/Xa), XCI (Xa/Xi), or XCI with erosion of silencing marks (Xa/Xi eroded). In this study, we analyzed the XCI status in KS derived hiPSCs. For hiPSC derivation, human dermal fibroblasts from one healthy female, two healthy male and two KS donors were transfected with episomal plasmids (pCXLE-hOCT3/4-shp53, pCXLE-hSK and pCXLE-hUL) and cultured on Laminin-521 in NutriStem medium. All hiPSC lines exhibit a patient specific karyotype, show no difference in pluripotency expression between healthy, KS and the hESC control line (HS980). All hiPSC lines had the potential to differentiate towards the three germ layers in vitro and in vivo. XCI status was assessed by H3K27me3 staining, which is a repressive methylation mark that accumulates on the Xi, and by RNA fluorescence in situ hybridization (FISH) for XIST, a long non coding RNA that covers the Xi. Both healthy male lines had no H3K27me3 accumulation and no XIST cloud in the nucleus, while the healthy female hiPSC line showed one area of H3K27me3 accumulation and one XIST cloud in most of the cells. Interestingly, the KS hiPSC lines consisted of three cell populations, showing either one, two or no H3K27me3 accumulation as well as one, two or no XIST clouds. So far two XIST clouds could only be observed in naïve hESCs and hiPSCs as well as preimplantation embryos, thus investigation of allele-specific X-linked gene expression will provide interesting information about XCI of primed KS hiPSCs contributing to a better understanding of XCI. Further, our KS iPSCs could be used to study the possible effect of XCI state for germ cell differentiation in vitro, providing new insights into germ cell development failures of KS patients.

T-3034

GENERATION OF GRNA LIBRARIES FOR CRISPR-BASED (EPI)GENOMIC SCREENS

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Increasing evidence suggests that chromatin marks play a crucial role in the regulation of gene expression, with several studies revealing significant correlation between chromatin marks and transcriptional regulation. However, it is still unclear, where a specific chromatin mark has to be located in order to be functionally active, and whether said regulation is strong enough to subsequently define and change cell identity. In our studies we use a modified DNA-nuclease, dCas9, as shuttle, for chromatin modifying enzymes. The dCas9-system uses short guide RNAs (gRNAs), that encode the genomic target. In our studies we use this system to target either specific genomic loci in candidate approaches, or a variety of genes in epigenomic screens, and subsequently investigate the effect of changed chromatin marks on the transcription of related genes. However, to expand current screening approaches to enable screens on an epigenome wide scale, more complex gRNA libraries are required. Here we describe approaches to generate high complexity libraries via either in silico sequence generation or, for virtually genome wide libraries, through controlled nuclease digest of input DNA. The possibility of subsequently cloning the acquired sequences into various plasmids enables for the generation transfectable or transducable gRNA libraries in a simple, time- and cost-effective procedure.

T-3036

UNDERSTANDING HUMAN CEREBRAL ORGANOID GENERATION EFFICIENCIES

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Three dimensional culture systems provide a physiologically relevant model for studying human brain development and disease. By directing the differentiation of human pluripotent stem cells (hPSCs) and supporting their intrinsic ability to self organize in three dimensions (3D), researchers have successfully generated organoids comprised of populations of cell types found in the brain; however, the efficiency of organoid generation from single cell populations is low and varies between different hPSC lines. Our findings suggest that initial pluripotent stem cell conditions could have an effect on cerebral organoid generation efficiencies. We will compare success rates of cerebral oganoid generation when establishing organoids from hPSCs in mouse embryonic fibroblast co-cultures compared to Matrigel and TeSR culture conditions. Additionally, we will screen various cell lines at different passages to analyze their ability to form organoids. ROCK inhibitor concentrations used in initial organoid seeding from single-cell populations are also a likely contributing factor to success in later stages of organoid development. We will test and analyze organoid generation with and without ROCK inhibitor when initially seeding organoids. Our research to better understand the culture conditions and factors influencing organoid generation can lead to more robust organoid generation systems and pave the way for high throughput development and disease modeling.

Funding Source: CIRM

T-3038

ENGINEERED CEREBELLUM FOR BRAIN REGENERATIVE MEDICINE

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Cerebellum is likely to become a major platform to investigate how making engineered whole cerebellum for brain regenerative medicine. A new approach to treatment involves the use of three-dimensional biological scaffolds made of allogeneic extracellular matrix. The studies described here illustrate the feasibility of engineering whole cerebellum in vitro when acellular matrices are combined with efficient recellularization strategies. These scaffolds can act as an inductive template for functional tissue and organ reconstruction after recellularisation with mouse fetal cerebellar progenitor cells. These cerebellar progenitor cell were deliver to a biologic scaffolds composed of mouse postnatal extracellular matrix (ECM) in vitro to construct remodeling of cerebellar tissues. We performed patch-clamp recordings to evaluate the electrophysiological phenotype of the cerebellar granule neurons after 50 days. Whole-cell patch-clamp recordings confirmed that the granule neuronal cells exhibited properties of functional mature neurons. About 65% (n=21 out of 32cells recorded) of the induced granule neurons fired mature action potentials in response to depolarizing current injection, and expressed the voltage-gated inward Na+ and outward K+ currents. The cerebellum scaffolds also showed the ability to differentiate progenitor cells into Purkinje's cells and astrocytes. This preliminary work demonstrates the biocompatibility of cerebellum scaffolds and supports the potential for engineered whole cerebellum for brain regenerative medicine

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T-3040

COMMERCIALIZATION OF REGENERATIVE MEDICINE TECHNOLOGIES

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Regenerative medicine (RM) has the potential to transform human health. In 2015, the RM market was estimated to be $4B and it is projected to grow to $10B by 2022. A collaborative project was undertaken with University of Toronto MBA candidates partnering with PhD candidates currently undertaking RM-related research. We sought to investigate whether RM technologies could be better commercialized by defining and elucidating the RM value chain. Interviews with key stakeholders and study of previously commercialized RM technologies was undertaken to investigate two hypotheses: (i) key components of the value chain exist; and (ii) value chains components are accessible and can be integrated. We found that a clear integration strategy ensures accessibility of value chain components and that coordinated integration of those components can help create synergies to unlock previously unrealized value. In summary, optimal coordination and leveraging of the RM value chain will lead to more successful commercialization in this space.

T-3042

IDENTIFICATION AND ASSESSMENT OF NOVEL BONE MORPHOGENIC PROTEIN RECEPTOR BINDING PEPTIDES IN OSTEOBLAST DIFFERENTIATION SIGNALING PATHWAY

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Bone morphogenetic proteins (BMPs) are part of the transforming growth factor-β superfamily and function as key regulators of cellular growth, differentiation, and tissue formation. While recombinant BMPs can be used to induce osteoblast differentiation, they are expensive to produce. In contrast, peptides can be efficiently produced in massive quantities through phage display. It would therefore be highly valuable to develop short peptides capable of mimicking BMPs which could be displayed on human-safe M13 phage. We have discovered 4 short, 12-mer peptide sequences (full sequences are not given for patent purposes) through a selective process called biopanning. These 12-mer peptides have been shown to bind the BMP receptors on live cells by immunofluorescence and bind the BMP receptors in an ELISA when genetically displayed on the p3 coat protein of M13 bacteriophage. In addition, the peptides were genetically displayed on the p8 (~2700 copies) coat protein of M13 bacteriophage to greatly increase the number of copies available for binding relative to the p3 (5 copies) coat protein. These newly engineered phages were incorporated into phage films having aligned ridge groove surface topographies. Human mesenchymal stem cells cultured on these films demonstrated varying levels of differentiation according to the peptide displayed as well as the concentration of the peptide. Differentiation has so far been confirmed by immunofluorescence of the marker proteins osteopontin and osteocalcin.

T-3044

ILOPROST SUPPORTS EARLY DEVELOPMENT OF IN VITRO-PRODUCED PORCINE EMBRYOS THROUGH ACTIVATION OF THE PHOSPHATIDYLINOSITOL 3-KINASE/AKT SIGNALLING PATHWAY

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Despite the several evidences concerning the presence of prostaglandin I2 (PGI2) in mammalian oviducts, its role in early embryonic development was largely unknown. Thus, the current study was carried out to examine the effect of PGI2 on in vitro developmental competence of porcine early embryos and underlying mechanism(s) by supplementing iloprost, a PGI2 analogue, into in vitro culture (IVC) medium. Especially, trophectoderm cell numbers were greatly increased, and cell survival was considerably improved in the blastocysts of 1 μM iloprost treatment group. Interestingly, Western blotting analysis showed that phosphorylation of Akt was markedly increased by treatment with 1 μM iloprost, suggesting the activation of PI3K signaling pathway. In addition, blastocyst formation rate, cell numbers and cellular survival were greatly reduced by Wortmannin, a potent PI3K inhibitor, which were significantly ameliorated by 1 μM iloprost treatment. Consistent with results from the IVF embryos, 1 μM iloprost improve the developmental competence in both parthenogenetically-activated and somatic-cell-nuclear-transferred embryos. Taken together, thμese results demonstrated that iloprost efficiently enhances the early embryonic development via Akt activation in pigs. Therefore, the current study strongly suggests that iloprost can be defined as a useful IVC supplement for massive production of porcine early embryos with high developmental competence.

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T-3046

THE CIRCULAR RNA CIRCB PARTICIPATE IN THE MOLECULAR CIRCUITRY CONTROLLING HUMAN PLURIPOTENCY

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Accumulating evidence indicates that circular RNAs (circRNAs) are highly prevalent in the human transcriptome. However, their involvement in human pluripotency remains elusive. Here, we identified a subset of circRNAs that are enriched in undifferentiated human embryonic stem cells (hESCs) and demonstrated that two circRNAs, circB and circC, are functionally associated with the pluripotent state. Mechanistically, we found that circB is enriched in the AGO2 complex and directly interacts with the microRNAs (miRNAs), miR-34a and miR-145, resulting in increased levels of these miRNA's targets and suppression of the hESC differentiation mediated by these two miRNAs. We further identified a set of hESC-enriched splicing factors and demonstrated that circB biogenesis in hESCs is promoted by the splicing factor ESRP1, whose expression is controlled by the core pluripotency-associated factors, OCT4 and NANOG. Collectively, these data indicate that circRNA serves as a miRNA "sponge" as part of the molecular circuitry that modulates human pluripotency and differentiation.

T-3048

PLURIPOTENCY AT THE MULTICELLULAR LEVEL

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Pluripotency refers to the capability of a single cell to generate all the cell types of the organism. However, it is not a single condition as different pluripotent states can be identified in vivo and in vitro, based on transcriptional, epigenetic and metabolic patterns. The biological significance of these states with respect to embryo morphogenesis remains unclear. We now show that exit from an unrestricted naïve pluripotent state is a pre-requisite for epithelialization in mouse and human embryonic stem cells and embryos. Naïve cells are able to polarize but fail to open a lumen and to establish the pro-amniotic cavity. Furthermore, our data show that lumen formation is transcriptionally controlled via expression of mucin proteins downstream of naïve pluripotency exit. The developmental arrest of mouse and human embryos upon deregulation of the pluripotency network supports the physiological relevance of these findings.

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T-3050

MOLECULAR REGULATION OF MESENCHYMAL STEM CELLS BY ARYL HYDROCARBON RECEPTOR LIGANDS

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Obesity is a major risk factor for development of type 2 diabetes and heart diseases. Adipose tissue regulates systemic metabolism through producing adipokines that regulate energy homeostasis. Epidemiological studies indicate increased incidence of type 2 diabetes after exposure to persistent organic pollutants (POPs). POPs accumulate in adipose tissues and activate the aryl hydrocarbon receptor (AhR). However, the mechanisms by which POPs alter metabolism remain poorly understood. Adipogenesis is a process by which mesenchymal stem cells (MSCs) differentiate into mature adipocytes. Limitations in adipogenesis and accumulation of ectopic lipid have significant roles in decreasing insulin sensitivity. Thus, we hypothesized that POPs contribute to systemic insulin resistance by lowering the rate of MSCs differentiation; the resulting large, poorly-functioning adipocytes will increase serum lipids and promote lipid deposition in other tissues. MSCs derived from mouse bone marrow were treated with POPs and levels of transcripts and proteins associated with adipocyte differentiation were determined by using quantitative PCR and western blot. Oil red O staining and content was performed to examine differentiation into mature adipocytes. Genes that promote adipogenesis including peroxisome proliferator-activated receptor gamma, fatty acid binding protein 4, and adiponectin were downregulated in mesenchymal stem cells treated with POPs. Moreover, accumulation of triglycerides was decreased after POPs treatment. Recombinant lentivirus vector-mediated AhR knockdown or treatment with the AhR antagonist blocked the effects of POPs on adipogenesis. These data suggest that activation of AhR by POPs increase risk of insulin resistance and type 2 diabetes by impairing adipogenesis. Reduced adipogenesis likely decreases adipocyte capacity to capture triglycerides from the blood. These effects

may disturb energy homeostasis and contribute to the development of metabolic syndrome.

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T-3052

ARCHITECTURAL MANIPULATION OF MULTIPOTENT LUNG PROGENITOR CELLS TO CONTROL CELL FATE CHOICE

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Chemical cues are used extensively to drive the directed differentiation of pluripotent stem cells toward lung epithelia. Emerging evidence suggests a role for mechanical cues in cell fate specification, however, engineering these cues to drive specification in lung remains unexplored. Human embryonic stem cell derived lung epithelia (NKX2.1+FOXA2+) in monolayer are dual positive (SOX2+SOX9+) lung progenitors (DPLP) and subsequently give rise to 3D branches in vitro with differential localization of SOX2 (proximal) and SOX9 (distal) protein. We hypothesized that exposure to developmentally relevant architecture would result in differences in fate choice. The tubular architecture of the lung during pseudoglandular and canalicular stages of development, was replicated using soft lithography to create 40-400μm diameter cylindrical cavities with a depth of 180μm. DPLPs formed single-cell lined tubes within engineered cavities of 100μm gelatin or PDMS cavities and became mostly SOX9+ (84.7±7.0% and 75.3±8.6%). In contrast, cells cultured for the same period on flat gelatin or PDMS were predominantly dual positive (95.0±5.1% and 80.4±4.6%). To ensure that decreased SOX2 expression was not due to architecture-created concentration gradients, DPLPs were grown on 100μm PDMS posts rather than cavities and were found to be primarily SOX9+ (80.2±5.6%). SOX9 single positive cells were modestly reduced in the presence of ROCK inhibitor H1152 suggesting that intracellular tension is partly responsible for decreasing SOX2 expression. Subsequent recovery and growth of SOX9+ cells generated within 100μm tubes on flat culture showed maintenance of SOX9 at day 1 (77.6%) but an expansion of dual positive cells after 10 days (65.3%). Recovered cells exposed to proximal or distal airway inducing conditions gained expression of distal epithelial markers (SP-B, LPCAT1) but not proximal markers (SOX2, p63, MUC5AC). In contrast, cells recovered from flat culture were capable of expressing both proximal and distal markers. Our study suggests that mechanical cues can significantly impact progenitor cell fate specification and thus their inclusion in directed differentiation protocols may lead to the generation of clinically relevant culture models and cell populations respectively.

Funding Source: CIHR Training Programme in Regenerative Medicine. McLaughlin Centre. The Henry White Kinnear Foundation.

T-3054

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO INSULIN-PRODUCING CELLS

Kim, Min Jung, Kim, Ji-Won, You, Young-Hye and Yoon, Kun-Ho
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The development of human iPSCs will open the opportunity for personalized medicine. This study focused on the importance of endodermal differentiation from human iPSCs in order to obtain functional pancreatic β-cells for source of transplantation to rescue the diabetes patients. We have developed a highly efficient step-wise protocol to direct insulin-producing cell from diabetes patient-specific iPS cell lines. For differentiation into insulin producing cells, we divided into 4 stages. We regulated the glucose concentration and used several small molecules in each steps and adenovirus for Pdx-1 overexpression in the stabilizing stage. In final step, we reaggregated the cells like an islet cluster. Interestingly, aggregated cells showed expression of insulin gene and insulin positive cells. To functional test, we performed glucose-stimulated insulin secretion. The insulin secretion was a statistically significantly increased by high glucose treatment in differentiated cells. We have confirmed differentiating ability of iPSC into insulin producing cells. Human iPS cells could be useful alternative sources in clinical applications with the generation of β-cell surrogates. Further studies would be required to optimize the protocols for differentiating into insulin-producing cells from iPSCs.

T-3056

GENERATION AND CHARACTERIZATION OF A MYH6 HUMAN EMBRYONIC STEM CELL REPORTER LINE USING CRISPR/CAS9 TECHNIQUE

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Atrial cardiomyocytes are essential for fluid homeostasis, ventricular filling, and survival. Dysfunction of cardiac atria play a key role in the pathogenesis of several

common heart diseases, such as atrial fibrillation. Human pluripotent stem cells have unique competence to generate atrial cardiomyocyte to model cardiac atria associated diseases. However, most current cardiomyocyte differentiation protocols generate mixed cell populations, which is makes it challenging for disease modeling. Here, we applied CRISPR/Cas9 technique to generate an α-MHC:mCherry human embryonic stem cell (hESC) knock-in reporter line. Further, we could isolate pure atrial myocytes using this reporter line. These α-MHC:mCherry positive cells express cardiac markers, and display atrial-like action potentials and global gene expression profiles. Hence, this α-MHC:mCherry hESC reporter line can provide us a useful tool to isolate pure atrial myocytes, which will pave a path to better understand cardiac atrial diseases.

Funding Source: Empire State Stem Cell Research Program (NYSTEM, #C028115).

T-3058

GENERATION AND CHARACTERIZATION OF PANCREATIC PROGENITOR CELLS DIFFERENTIATED FROM INDUCED PLURIPOTENT STEM CELLS REPROGRAMMED FROM ADULT HUMAN BETA CELLS

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Beta-cell replacement therapy is considered the optimal treatment for type 1 diabetes, however it is severely limited by the shortage of human organ donors. Generation of insulin-secreting cells from pluripotent stem cells represents a promising approach for providing an abundant source of cells for beta-cell replacement therapy. However, differentiation of pluripotent stem cells into mature beta cells is difficult to reproduce in multiple pluripotent stem cell lines. Our previous work has shown that induced pluripotent stem cells reprogrammed from adult human islet beta cells (termed BiPSCs) maintain an open chromatin structure in genes expressed in beta cells. This epigenetic memory may be responsible for the enhanced propensity of BiPSCs to spontaneously differentiate into insulin-producing cells in vitro and in vivo, compared with fibroblast-derived iPSCs (FiPSCs). When subjected to a stepwise protocol for in-vitro differentiation of pluripotent stem cells into beta cells, BiPSCs from 3 different human donors showed a reproducible enhanced differentiation capacity as early as the first stage of the differentiation (definitive endoderm, DE cells). BiPSCs gave rise to a higher percent of DE cells than FiPSCs, and showed elevated transcript levels of DE cell-specific markers SOX17, FOXA2 and CXCR4. Moreover, at a later differentiation stage (pancreatic progenitor, PP cells), BiPSCs-derived cells expressed higher levels of PDX1 and NKX6-1 transcripts, encoding beta-cell transcription factors, compared with FiPSCs-derived cells. These results demonstrate the enhanced and reproducible in-vitro differentiation capacity of BiPSCs into progenitor stages of the beta-cell lineage, compared with pluripotent stem cells from other sources, and suggest that BiPSCs may serve as a superior source for modeling beta-cell development and disease, as well as cell replacement therapy.

T-3060

MODELING MICROCEPHALY IN VITRO USING A TRIDIMENSIONAL NEURAL NETWORK

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Microcephaly is a neurological condition, resulting in patients having a small head circumference, intellectual impairment and brain anatomical defects. A pre-requisite for achieving a better understanding of the cellular events that contribute to the striking expansion of the human cerebral cortex is to elucidate cell-division mechanisms, which likely go awry in microcephaly. We decided to focus on one particular protein, KNL1, which plays a central role in kinetochore assembly and function during mitosis, and mutations in this gene are found in microcephalic patient. We employed CRISPR/Cas9-mediated genome engineering to generate isogenic human pluripotent stem cells bearing KNL1 patient mutation (KNL1 p. M2041I) and used SMAD-inhibition protocol to make neural progenitors (NPs). NPs were then differentiated in two- dimensional and three-dimensional neuronal cultures. The characterization of KNL1 p. M2041I NPs revealed reduced KNL1 expression, increased cell death, decreased cell growth and prolonged mitosis. At 4 weeks of neuronal differentiation, cell-surface markers revealed the presence of neurons and astrocytes in KNL1 p. M2041I, whereas these populations appear only at 6 weeks in controls. The combination of these phenotypes could lead to microcephaly, consistent with three-dimensional neuronal cultures, in which KNL1 p. M2041I neurospheroids were significantly smaller. We used KNL1 shRNA knock-down in NPs and we obtained a premature neural differentiation phenotype and a reduced size of neurospheroids. Finally, we rescued the phenotype by over-expressing KNL1 in KNL1 p. M2041I. We showed that KNL1 knock down by the patient mutation or by shRNA lead to premature differentiation and result into smaller neurospheroids. Our results highlight the importance of KNL1 underlying the pathogenesis of cell division-related microcephaly. This study provides insights into the molecular mechanisms

of mitotic regulation in human neural development and corticogenesis.

Funding Source: Supported by a Jerome and Florence Brill Graduate Student Fellowship

T-3062

GORLIN SYNDROME DERIVED NEUROEPITHELIAL STEM CELLS: A MODEL FOR MEDULLOBLASTOMA

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This study seeks to validate a new medulloblastoma stem cell model. Medulloblastomas are the most frequent malignant intracranial tumors in children, originating in the cerebellum. Hyperactivation of the Sonic Hedgehog (SHH) cell growth signaling pathway is the cause of 25% of medulloblastomas, either due to a Ptch1 mutation or to the mutation of other genes that activate the cascade. Gorlin syndrome is a hereditary condition in which patients harbor a Ptch1 germ-line mutation that causes aberrant SHH signaling and predisposes to ectodermal tumors, such as nevoid basal cell carcinoma and medulloblastoma. In this study we used neuroepithelial stem cells (NES cells) generated from reprogrammed keratinocytes of Gorlin syndrome patients. Four main conclusions from this study support the validity of the model: (1) Primary cilia –the cell organelles where SHH signaling pathway takes place- are present in neuroepithelial stem cells, which indicates that the conduit for SHH signaling is present. (2) A higher percentage (14,01%) of Gorlin syndrome-derived NES cells (Ptch1+/-) display primary cilia when compared to (3,86%) control cells derived from healthy patients, ($p < 0,001$). The percentage of primary cilia is significantly higher (20,22%, $p < 0,001$) in NES cells injected into mice and later subcultured, possibly due to the selection of the more aggressive cell populations. (3) A significant upregulation of the SHH signaling pathway in Ptch1+/- cells was observed in gene expression analysis of GLI2, a SHH transcription factor, and CCND1, a SHH proliferation target gene. (4) Finally, when injected into mice in in vivo studies, Ptch1+/- cells give rise to tumors, while control cells do not. These findings support the validity of our novel Gorlin syndrome derived NES cells model as a SHH medulloblastoma stem cell model, which could help understand tumor initiation and be used in screening for new treatments.

Funding Source: Karolinska Institutet and Amgen Foundation.

T-3064

TRANSCRIPTOMIC AND NEUROPHYSIOLOGIC ANALYSIS FROM HUMAN IPSCS REVEALS CONVERGENT PATHOBIOLOGY IN IDIOPATHIC AUTISM

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Autism spectrum disorders (ASDs) are a phenotypically, etiologically and genetically complex group of neurodevelopmental conditions. A large proportion of genes associated with ASD are functionally interconnected through pathways that regulate a wide range of neurobiological processes during brain development. The aim of this study is to identify key differences in the function of important neurobiological processes, and in the expression patterns of molecular gene networks that regulate them, both in a temporal and neural–specific manner. This data is essential to better understanding the effect ASD has on neuronal development. Defining common deregulated pathways and biological processes in the pathobiology of idiopathic ASD constitutes an important resource for investigations studying biomarkers and therapies that will eventually play an important role in providing a better quality of life for individuals affected by ASD. We examined the transcriptional differences between cortical neurons from patients with idiopathic ASD and healthy unaffected control individuals over the course of their in vitro development. We derived iPSCs from peripheral blood mononuclear cells (PBMCs) from idiopathic ASD individuals and unrelated male controls and differentiated them into cortical neurons over a 135 day time course. Transcriptional analyses of ASD and control neurons at culture days 35, 85, and 135 showed ASD-specific molecular phenotypes mainly affecting pathways/networks involved in neuronal differentiation, the cytoskeletal matrix structure formation regionalization, patterning, DNA and RNA metabolism. Concurrently, networks of neurons were interrogated with multi-electrode array (MEA) recordings, measurements of calcium transients, cell migration assays, and morphological analyses. ASD individuals demonstrated significantly decreased network spiking activity from MEA recordings decreased numbers of calcium transients, significant differences in measures of neurite morphology, and decreased cell migration. In our cohort there are early deficits in network physiology

that converge with our transcriptomic analyses. Taken together, this suggests that for our cohort of individuals with idiopathic ASD, there are convergent pathophysiological processes.

Funding Source: HIAS15004 2014-2018 Hussman Foundation Pilot Grant (M.W.N)

T-3066

THERAPEUTIC CORRECTION OF DYSTROPHIN GENE IN A HUMAN PLURIPOTENT DMD DISEASE MODEL

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Duchene muscular dystrophy (DMD) is a genetic disease caused by defects in the dystrophin-glycoprotein complex that stabilizes skeletal muscle. DMD is caused by mutations along the dystrophin gene that result in a frame shift leading to a truncated dysfunctional protein. Exon 51 deletion of the DMD gene is a therapeutic approach that can restore the dystrophin reading frame in 13% of the DMD cases. To model this therapeutic approach, we utilized CRISPR/Cas9 technology to delete exon 52 in wild-type human iPSC line and create a DMD model line suitable for therapeutic correction using exon 51 deletion. Differentiation of DMD iPSCs into skeletal muscle using a novel differentiation protocol, showed the absence of dystrophin protein. To model the therapeutic correction of DMD, we utilized a dual gRNA CRISPR/Cas9 nuclease approach to delete exon 51 in the DMD model line which restored the dystrophin reading frame. Skeletal myotubes derived from the reframed DMD human iPSC lines showed restoration of dystrophin protein expression. This work demonstrates the feasibility of using CRISPR/Cas9 to correct the reading frame in a portion of DMD patients.

T-3068

SUPER-OBESE PATIENT IPSC-DERIVED HYPOTHALAMIC NEUROPEPTIDERGIC NEURONS SHOW DYSREGULATED OBESITY ENDOCRINE PATHWAYS

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The arcuate nucleus of the hypothalamus contains neurons responsible for feeding. They control metabolic processes by secretion of neuropeptides in response to hunger and satiety signals from the gastrointestinal tract, pancreas and adipose tissue. Here we aim at addressing the challenge of non-availability of developing human brain/hypothalamic tissues for study. To bridge this gap, we generated hiPSCs from lymphoblasts of super obese (body mass index (BMI) > 50 (n = 4)) and normal subjects (BMI < 25 (n = 5)) and differentiated them into hypothalamic neuronal cultures (iHTNs) specifically those expressing pro-opiomelanocortin (POMC), agouti-related peptide (AgRP), cocaine and amphetamine regulating transcript (CART) and neurons capable of secreting neuropeptides such as neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (MSH). Of the total number of neurons (approx.75% of cells in culture), we saw 42% MSH, 44% NPY, 28% AgRP and 20% POMC/CART neurons. Bioinformatics analyses of transcriptomics from day 40 differentiated iHTNs reveal that these neuronal cultures maintain predominantly a fetal hypothalamic identity. We further show that these neurons are capable of relevantly responding to physiological (exogenous) metabolic stimuli e.g. ghrelin, leptin and peptide YY. Notably, transcriptomics of iHTNs obtained from super obese individuals retained signatures of obesity showing specific dysregulation of obesity-related genes such as neuronal growth regulator 1 (NEGR1) and secretogranin 3 (SCG3). Ingenuity Pathway Analysis also highlighted dysregulation of several obesity-related metabolic pathways like leptin signaling, GPCR-mediated enteroendocrine signaling, ER stress pathway and inflammasome pathway in super obese iHTNs when compared to controls. The methods and characterization of iHTNs described here, could serve as a cellular platform for modeling and testing gene-environment interactions involved in metabolic diseases and obesity.

T-3070

GENERATION OF BLIND MOLE-RAT INDUCED PLURIPOTENT STEM CELLS AND THEIR CONTRIBUTION TO INTERSPECIES CHIMERA

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Blind mole-rats (BMRs, Spalax sp.) are rat-sized rodents. These subterranean cancer-resistant animals show maximum lifespan exceeding 20 years compared to 4 years for rats and mice. We report the generation of blind mole-rat induced pluripotent stem cells (BMR iPSCs) from lung fibroblasts of these animals by forced expression of mouse OSKM factors. The iPSC colonies were shown to express pluripotency markers, including alkaline phosphatase, Oct4, Sox2, Nanog, SSEA1, and E-cadherin. The ability of iPSCs to differentiate in vitro was confirmed by embryoid bodies formation as well as their spontaneous differentiation to the derivatives of the three germ layers. In addition, the BMR iPSCs readily formed teratoma upon injection into the renal capsules of immunodeficient mice. We further succeeded in generating viable interspecies chimera between the BMR and mouse following injection of BMR iPSCs into mouse blastocysts. The BMR cells could overcome the evolutionary distance of 45 million years in contributing to chimera formation. The resulting model allows examining critical questions in cancer and aging fields, most notably contribution of cells from another species to mouse longevity and cancer resistance.

T-3072

TRANSCRIPTIONAL CAPACITY LIMITS CELLULAR REPROGRAMMING

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Cellular reprogramming initiates a massive transcriptional realignment, inducing genome-wide topological strain and breaks in DNA. When transcription exceeds the capacity of DNA repair machinery to resolve topological tangles and DNA breaks, transcription stalls, retarding reprogramming processes. Previous work to elucidate the rules of conversion identified that "privileged" fast-cycling cells convert most efficiently. Using a combination of chemical and genetic methods to perturb cell cycle rate, we profiled cells early in conversion to identify mechanisms that promote privilege. Increasing the cell cycle rate upregulates the DNA-repair pathway leading to sustained high levels of transcription as measured by EU incorporation. Increasing transcription alone via overexpression of an hRAS mutant that globally increases transcription was insufficient to enhance conversion. Knockdown of topoisomerase Top2A, a vital component of elongation and DNA repair during transcription, reduced the conversion advantage of fast cycling cells. Transcriptional capacity, the combination of transcription rate and DNA repair rate, limits conversion and mediates competition between donor and alternate gene regulatory networks (GRNs). By single-cell transcriptional profiling, we identified that expression of the donor cell transcription factors (TFs) limits adoption of the alternate fate in a dose-dependent manner. Expanding transcriptional capacity reduces competition from the donor GRN and accelerates conversion. Greater transcriptional capacity allows cells to maintain higher levels of transcription during conversion and generate more mature identities across a range of conversion protocols and species. In particular, conversion of human adult primary fibroblasts to induced motor neurons (iMNs) increases ten-fold. In the iMN system, our enhanced conversion protocol increases transcriptional fidelity to primary MNs, improves electrophysiological maturity, and preserves sensitivity to disease stimuli. Expanding cellular processing capacity increases the power of transcription factor-mediated programs to compete with established GRNs and mediate robust conversion to new cellular identities.

Funding Source: NIH NRSA (F32) Ruth L. Kirschstein Postdoctoral Fellowship

T-3074

DIRECT INDUCTION OF MOUSE NEURAL PROGENITOR CELLS TRANSIENTLY PASSES THROUGH A PARTIALLY REPROGRAMMED STATE

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The generation of functional neural progenitor cells (NPCs) holds great promise for both research and clinical applications in neurodegenerative diseases. Traditionally, NPCs are derived from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), or NPCs can be directly converted from somatic cells by sets of transcription factors or by a combination of chemical cocktails and hypoxia. However, the ethical issues of ESCs, the risk of tumorigenesis from iPSCs and transgenic integration from exogenous genes as well

as complicated manipulation and time-consuming of ciNPCs limit the applications of these strategies. Here, we describe a novel method for generating growth factor-induced neural progenitor cells (giNPCs) from mouse embryonic and adult fibroblasts by using inductive and/or permissive signaling culture conditions. These giNPCs closely resemble brain-derived NPCs in terms of transcription networks and neural lineage differentiation potentials. Moreover, this somatic cell to NPC induction is a gradual process that includes initiation, intermediate, maturation and stabilization stages. Importantly, gene expression and histone modification analyses further indicate a partially reprogrammed state during the generation process of induced NPC, in which lineage specific genes and pluripotency-associated genes are transiently activated. Our study therefore describes the potential safety problems that also exist in the transgene-free direct induction strategy and highlights the importance of excluding the possibility of residual partially reprogrammed and/or teratoma-like cells from the generated NPCs for future clinical trials.

Funding Source: This project was supported by the National Natural Science Foundation of China and the Ministry of Science and Technology of China.

T-3076

CONTROLLED SELF-ASSEMBLY OF STEM CELL AGGREGATES INSTRUCTS PLURIPOTENCY AND LINEAGE BIAS

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Recent studies show that three-dimensional (3D) aggregates of stem/progenitor cells can self-organize into organoids resembling native tissues in their structure and function. A common starting material for organoid formation is stem cell aggregates, which can be formed via several methods including spontaneous aggregation, gravitational sedimentation, and forced centrifugation into microwells. However, conventional methods offer little control over parameters associated with the aggregation process (e.g., kinetics) and the structural properties of resulting aggregates (e.g., cell density, porosity), which limits the identification of optimized conditions for organoid formation. Here we developed chemically defined labile substrates to control spatiotemporal dynamics of a cell aggregate self-assembly (SA) process. Our approach enables the enhanced-throughput generation of SA aggregates of many cell types, including human pluripotent stem cells (hPSCs) and hPSC-derived hepatocytes and neural progenitors. By comparing SA aggregates to those formed via conventional methods, we found that distinct aggregation methods and kinetics result in divergent gene expression profiles in multiple biological contexts including spontaneous embryoid body (EB) differentiation. For example, conventional EBs were associated with a rapid decline in expression of pluripotency markers Oct4 and Nanog, while the loss of these markers was delayed in SA-EBs. Conventional methods promoted ectoderm fates in differentiating EBs while SA-EBs expressed genes indicative of mesoderm and endoderm fates; however, changing aggregation kinetics alone significantly influenced lineage-specific gene expression in SA-EBs. Furthermore, we observed stark differences in structural properties between methods of aggregation; conventional EBs were dense and compacted while SA-EBs exhibited low cell density and a highly porous structure. Our results indicate that aggregation parameters influence stem cell differentiation trajectory, potentially by dictating aggregate structural properties. Future studies will aim to better understand implications of these properties for applications of cell aggregates in stem cell biomanufacturing and the generation of organoids.

Funding Source: This work was supported by funding from the National Institutes of Health (Biotechnology Training Program NIGMS 5 T32-GM08349 to A.W.X. and A.S.K.) and the National Science Foundation (DGE-1256259 to A.W.X. and A.S.K.).

T-3078

CHARACTERISATION OF SEVEN NEW MONOCLONAL ANTIBODIES TO DEFINED CELL SURFACE PROTEINS ON HUMAN PLURIPOTENT STEM CELLS

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The ability of human pluripotent stem cells (hPSCs) to undergo indefinite self-renewal and differentiation into all cell types of the 3 embryonic germ layers has revolutionised regenerative medicine. The availability of well-characterised and validated monoclonal antibodies (mAbs) detecting cell surface proteins on hPSCs is crucial for further development of this growing industry. We recently generated a panel of monoclonal antibodies to the following human proteins based on our previous target identification; CUB domain containing protein 1 (CDCP1), platelet F11 receptor (F11R), desmoglein 2 (DSG2), cadherin 3 (CDH3), neuroligin 4X-linked (NLGN4X) and protocadherin 1 (hPCDH1) and G protein-coupled receptor 64 isoform 4 (GPR64). Our findings

demonstrate that these antibodies are valuable tools for studying naive and primed state hPSCs in addition to applications for somatic cell types. To extend our recently published study, we have directly conjugated each antibody to either Alexa Fluor 488, Alexa Fluor 647, R-phycoerythrin (PE), or allophycocyanin (APC). Using flow cytometry and immunocytochemistry techniques, we demonstrate that there is a high correlation of staining between the conjugated monoclonal antibodies and OCT3/4, TRA-1-60, and SSEA-4. These results provide insights into expression levels of these cell-surface proteins on differentiating hPSCs. We anticipate that the panel of antibodies reported here will provide tools to further investigate the cellular mechanisms underlying pluripotency in hPSCs. The mAbs reported here should accelerate the investigation of the nature of pluripotency, and enable development of robust cell separation and tracing technologies to enrich or deplete for hPSCs and other human stem and somatic cell types.

T-3080

EFFICIENT DELIVERY OF CAS9 PROTEIN AND GUIDE RNA USING 30KC19 PROTEIN FOR CRISPR-BASED GENOME EDITING

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The type II CRISPR system is an adaptive immune response consisting of Cas9 protein and guide RNA in bacteria and has been utilized as an alternative genome editing tool. In this system, it has been concerned that virus mediated-gene delivery for the expression of Cas9 and guide RNA may lead to unwanted genomic modification of host cells. Here, we produced recombinant Cas9 fused with cell-penetrating 30Kc19 protein. The recombinant 30Kc19-Cas9 fusion protein was produced by E.Coli and purified by histidine-tag affinity chromatography. The conjugation of 30Kc19 did not affect the expression level and yield after purification. Compared with Cas9, 30Kc19-Cas9 showed similar thermal stability. We also demonstrated that 30Kc19-Cas9 can cleave a specific DNA sequence with single guide RNA (sgRNA) by using an in vitro DNA cleavage assay. To investigate whether the 30Kc19-Cas9 can cleave a target sequence within human cells, we treated 30Kc19-Cas9 with sgRNA targeting the EGFP gene to the stably EGFP-expressing HEK293 cells. As a result, the intracellular fluorescence of EGFP was significantly reduced. In addition, we demonstrated that the treatment of 30Kc19-Cas9 and sgRNA can give rise to small insertion and deletions (indels) in a target genomic locus of human induced pluripotent stem cells by using T7 endonuclease assay. These results suggest that 30Kc19-Cas9 protein could be used as a great tool for genome editing of human pluripotent stem cells.

T-3082

RNA-SEQ CELLNET IDENTIFIES CELL FATE ALTERING MUTATIONS, DISRUPTED TRANSCRIPTIONAL CASCADES, AND ACTS AS A QUALITY CONTROL TO IMPROVE POWER OF DISEASE IPS STUDIES

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Patient specific iPS have transformed our ability to model human disease. These studies generally compare disease and control iPSC-derived lineages functionally, phenotypically, and molecularly. However, variation in the in vitro differentiation potential of lines that is not related to the disease can confound these investigations. Previously, we developed CellNet, a computational platform that assesses the fidelity of cell fate engineering by measuring the establishment of cell type specific gene regulatory networks from gene expression data. Here, we applied our RNA-Seq version of CellNet to 24 published disease iPS studies to determine whether there are systematic differences between disease and control iPS in the undifferentiated state and in differentiated lineages. While disease- and control iPS were indistinguishable in the pluripotent state, several iPS-derived differentiated lineages were significantly different in lineage status (as read out by CellNet). Because some disease causing mutations may alter cell fate, we devised an algorithm that scores samples according to the extent to which their lineage status is consistent with known fate-altering mutations. We have made RNA-Seq CellNet accessible as a user friendly web application that will allow researchers to upload their raw RNA-Seq data and receive both gene expression estimates and CellNet analysis results. We have also included an option in this pipeline that will allow researchers to select from hundreds of processed unaffected iPS and iPS-derived profiles for inclusion in their analysis. Collectively, these resources will enable researchers to distinguish uninteresting differentiation bias from cell fate altering mutations, to identify problematic control lines, and to supplement their own studies with a panel of equivalently-processed unaffected profiles.

T-3084

HIGH-RESOLUTION FUNCTIONAL IMAGING OF CARDIOMYOCYTES DERIVED FROM EMBRYONIC STEM CELL SPHEROIDS

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Embryonic stem cell tests have been used for safety assessment of compounds in vitro to reduce the use of animals. Hanging-drop technology can be used for culturing embryonic stems cells and provides embryoid bodies or 3D spheroids that mimic in vivo-like tissue structures. In this work, we show that successful differentiation of embryoid bodies into beating cardiomyocytes can be reliably detected by using high-density microelectrode arrays. High-resolution electrical imaging allows for improved sensitivity in detecting beating cells, for localization of ECG-like waveforms in QT interval measurements, and for tracking of cardiac action potential propagation. The latter parameters can be used as functional endpoints for evaluating the effects of compounds on cardiomyocyte activity.

FRIDAY, JUNE 16, 2017

LATE BREAKING POSTER SESSION III-ODD 18:00 – 19:00

F-3001

ADIPOSE-DERIVED STROMAL VASCULAR FRACTION: CHARACTERIZATION OF THE CELLULAR COMPONENTS AND THERAPEUTIC POTENTIAL IN A MURINE ARTICULAR INJURY MODEL

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The stromal vascular fraction (SVF), a collection of mesenchymal stem cells (MSCs), endothelial and hematopoietic stem/progenitor, and immune cells enriched from adipose tissue, has garnered interest as a therapeutic in regenerative medicine. Perhaps faster than any other cell preparation, SVF is making its way to clinics worldwide, while critical preclinical research needed to establish SVF safety, efficacy and optimal, standardized clinical procedures are still under progress. In here, we addressed two aspects of SVF biology: cellular composition and safety/efficacy profile. We characterize the stem/progenitor cell population present in enzymatically digested lipoaspirates, including the effects of different bio-metric parameters and co-morbidities on its cell composition. Next, we assess its safety and therapeutic potential compared to MSCs isolated from the same patient in a murine model of articular injury. Our results show that although age or body mass indexes (BMI) do not affect numbers of isolated nucleated cells, such parameters do impact its cellular composition. In particular, while the frequency of Tregs (CD4+CD127+) and M2 macrophages (CD14+CD163+) decreased with age, it positively correlated with increased BMI. Importantly, the frequency of MSCs (CD31-CD45- CD235a-CD34+) in the isolated SVF inversely correlates with increase BMI. Intra-articular injection of SVF, like MSCs, showed a safe profile, without any adverse effects or neoplasia. Functionally, repeated measurements on a force plate altimeter revealed that injured mice traveled lesser distances when compared to sham animals, and treatment with either MSC or SVF significantly rescued this phenotype. These results highlight the importance of donor variations in SVF preparations, which in turn should affect treatment dose, rationales and efficacy markers. In addition, the study here provides pre-clinical evidence for the safety and feasibility of utilizing SVF in articular injuries in a clinical setting.

F-3003

ASB2-DEPENDENT PROTEOLYSIS CONTROLS MAMMALIAN CARDIOMYOCYTE DEVELOPMENT

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The postnatal heart has very minimal intrinsic regenerative capacity. As such, cardiac stress often results in extensive cardiomycote death that can ultimately lead to heart failure, rendering cardiovascular defects a leading cause of death worldwide. A major approach for heart regeneration is the use of embryonic stem cells (ESCs). However, this is limited by the ability to achieve functional mature cells; thus, the need to understand the extensive cellular processes that govern proper heart formation and maturation. The Ubiquitin-Proteasome System (UPS) is important in regulating protein turnover during organ development but its role in the mammalian heart remains unclear. We have identified that a specificity subunit of an E3 Ubiquitin

ligase (Asb2) is specific for the cardiomyogenic lineage. Asb2 was previously reported to control skeletal myogenesis and hematopoiesis through targeting filamin proteins (FlnA, B and C), actin-binding proteins essential for cytoskeleton stabilization. In our current study, we show that Asb2 is highly expressed in ESC-derived cardiac progenitors and cardiomyocytes. To investigate the role of Asb2 and UPS dependent proteolysis in heart formation, we generated two cardiac-specific murine knockouts (KOs): NkxCre.Asb2-/- and Mef2cCre.Asb2-/- (deleting Asb2 in early cardiomyocyte progenitors and anterior heart field progenitors, respectively). Both KOs are embryonic lethal with pericardial edema. We used tissue clarifying and confocal microscopy to define the morphological defects of Asb2 null hearts. Moreover, we found that FlnA is overexpressed in the hearts of these mice and its deletion therein partially rescues their lethality. Using transcriptomic analysis on Asb2-null e9.5 hearts, we also identified novel potential Asb2 targets in the heart. Finally, to understand the role of Asb2 in human cardiomyocytes differentiation and function, we used CRISPR/Cas9 genome editing to generate Asb2-null human induced pluripotent stem cells. Collectively, our study provides novel mechanistic understanding of the UPS role in cardiac development, myocardial function, and disease pathogenesis. This enhances our knowledge of cardiac cell differentiation and maturation for the use of ESC-derived cardiomyocytes as potential therapeutic approach for cardiac repair.

F-3005

LNK IS AN IMPORTANT MODULATOR OF INSULIN-LIKE GROWTH FACTOR-1/AKT/ PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-GAMMA AXIS DURING ADIPOGENESIS OF MESENCHYMAL STEM CELLS

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Adipogenic differentiation of mesenchymal stem cells (MSCs) is critical for metabolic homeostasis and nutrient signaling during development. However, limited information is available on the pivotal modulators of adipogenic differentiation of MSCs. Adaptor protein Lnk (Src homology 2B3 [SH2B3]), which belongs to a family of SH2-containing proteins, modulates the bioactivities of different stem cells, including hematopoietic stem cells and endothelial progenitor cells. In this study, we investigated whether an interaction between insulin-like growth factor-1 receptor (IGF-1R) and Lnk regulated IGF-1-induced adipogenic differentiation of MSCs. We found that wild-type MSCs showed greater adipogenic differentiation potential than Lnk–/– MSCs. An ex vivo adipogenic differentiation assay showed that Lnk–/– MSCs had decreased adipogenic differentiation potential compared with wild-type MSCs. Interestingly, we found that Lnk formed a complex with IGF-1R and that IGF-1 induced the dissociation of this complex. In addition, we observed that IGF-1-induced increase in the phosphorylation of Akt and mammalian target of rapamycin was triggered by the dissociation of the IGF-1R–Lnk complex. Expression levels of a pivotal transcription factor peroxisome proliferatoractivated receptor gamma (PPAR-γ) and its adipogenic target genes (LPL and FABP4) significantly decreased in Lnk–/– MSCs. These results suggested that Lnk adaptor protein regulated the adipogenesis of MSCs through the IGF-1/Akt/PPAR-γ pathway.

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F-3007

FLI1 IS ESSENTIAL FOR THE MAINTENANCE OF HEMATOPOIETIC STEM CELL HOMEOSTASIS AND FUNCTION

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The ETS family of transcription factors is known to play an essential role in hematopoietic and vascular development. One such factor that is widely expressed in all vascular beds and almost all hematopoietic lineages, extending from long term hematopoietic stem cells to terminally differentiated peripheral blood cells, is Fli1. Global deletion of Fli1 leads to embryonic lethality at E12.5 due to dramatic hemorrhaging caused by poor vascular integrity and platelet dysfunction. The role of Fli1 in adults has been explored in several different cells types ranging from megakaryocytes to B and T cells. It has also been implicated in transcriptional regulation of hematopoietic stem and progenitor cells through combinatorial analysis, however its exact contribution to stem cell maintenance and function remains unclear. We hypothesized that Fli1 plays a dual role in regulating both the seed and the soil – specifically hematopoietic stem cell (HSC) function in a cell autonomous manner as well as the niche required for nurturing these cells. In this study, we focused our attention on the cell autonomous function of Fli1. We found that global deletion of Fli1 leads to lethality as a result of complete peripheral blood failure in addition to aberrant vasculature. Using the cre-lox system and various transplantation strategies, we identify Fli1 as one of the critical regulators of adult hematopoietic stem cell function. Specific deletion of Fli1 in the hematopoietic compartment alone is sufficient to

induce significant reduction in peripheral blood counts, accompanied by hemorrhage, resulting in lethality. On further inspection, Fli1-/- HSCs are unable to expand ex vivo or engraft in a competitive setting. Additionally, Fli1 is essential for hematopoietic reconstitution post radiation and its deletion abolishes the ability of stem cells to reconstitute the bone marrow and contribute to peripheral blood lineages. Taken together, our results indicate that Fli1 is required for maintenance of HSC homeostasis and function, making it one of the unique transcription factors to play a critical role in HSC function in adults as well as during development.

F-3011

DISEASE MODELING AND MECHANISM DISSECTION WITH HUMAN PLURIPOTENT STEM CELLS AND HUMANIZED MICE

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Human pluripotent stem cells provide a platform to model human disease. However, for complex disease such as type 1 diabetes (T1D), it is challenging for in vitro culture alone to provide novel insights into disease mechanisms. We propose to create humanized mice to overcome these limitations. By transplantation of stem-cell-derived β-cells (SC-β), we generated human pancreatic islet-like tissue in mouse pancreas. The engrafted cells expressed markers associated with functional maturity of β-cells. Human insulin levels similar to fasting insulin levels in humans were detected in transplanted mice for many months after engraftment. Engrafted human cells formed vascularized islet structures that harbored human pancreatic endocrine cells and showed structural characteristics of human islets. These results suggest that transplanted human SC-β cells can generate tissues with many attributes of human islets. Taken together, these results indicate that this approach combining stem cell engineering and humanized mouse models could provide mechanistic insights to the pathology of T1D and potentially other disease.

F-3013

THE MICRORNA-200 FAMILY REGULATES MURINE HAIR FOLLICLE DEVELOPMENT BY MODULATING CRITICAL SIGNALING PATHWAYS AND CELLULAR PROCESSES

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The microRNA-200 family is highly enriched in mammalian epithelial tissues, and has attracted increasing attention for its role in epithelial cancers. However, the function of this miRNA family during mammalian development is less well understood. To probe the functions of these miRNAs in an endogenous, developmental context, we sought to identify high-confidence targets for the family using a method to directly capture the miRNA-mRNA/Ago complex in murine skin. These direct physical interactions, combined with RNA-seq, revealed that miR-200s targets are involved in signaling pathways important for development, as well as in cell adhesion, migration and proliferation. Using in situ hybridization, we determined that the miR-200s are expressed within murine skin, and that their expression is further elevated in developing hair follicle progenitors. To interrogate whether these miRNAs function in hair follicle morphogenesis, we generated a skin-specific inducible mouse model of the miR-200b cluster (miR-200a, miR-200b and miR-429), as well as a skin-specific loss-of-function model of all five family members. Either forced expression or loss of function of the miR-200s in the basal epidermis and hair follicle progenitors leads to defects in hair follicle development, revealing a significant role for these miRNAs in the developing hair follicle. In addition, when we examined primary cultured keratinocytes with over-expression or loss of miR-200s, we observed concurrent control of proliferation, cell adhesion, and migration. Taken together, our study reveals a critical role for the miR-200s in regulating hair follicle development through targets involved in signaling pathways and cellular processes.

Funding Source: This project was funded by NIH F31 AR066463-01A1 to J.H.

F-3015

WNT/B-CATENIN SIGNALING IN THE REGULATION OF MURINE NEPHRON PROGENITOR CELL FATE

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Formation of a functional kidney depends on the proper balance between renewal and differentiation of nephron progenitor cells. Failure to achieve this balance can lead to kidney failure or stem cell tumors. For several years, we have known that signals from an epithelial structure known as the ureteric bud were essential for maintaining this balance. More recently it was discovered that one molecule, Wnt9b, signals via beta-catenin to promote both renewal and differentiation of the nephron progenitor cells. How one ligand signaling through one transcription factor can promote two seemingly contradictory cellular processes has remained unclear. We hypothesized that Wnt9b regulated NPC fate through a concentration gradient. Through genetic and pharmacological experiments I have determined that Wnt9b/B-catenin signal is sufficient to promote both renewal and differentiation. More importantly, graded levels of Wnt9b and B-catenin activity are sufficient to promote these two disparate responses with low levels fostering progenitor renewal and high levels driving differentiation. However, varied response to Wnt signaling occurs in the absence of an obvious Wnt ligand gradient. Instead, we find that signals emanating from the adjacent stroma modulate beta-catenin activity. Although further work needs to be conducted to elucidate the stromal signals that regulate Wnt/B-catenin signaling, our results provide insight into how Wnt9b regulates distinct target genes that balance nephron progenitor renewal and differentiation.

F-3017

A NOTCH POSITIVE FEEDBACK CONTROLLING INTESTINAL STEM CELL NICHE PATTERNING

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The intestinal epithelium is the fastest regenerative tissue in the body, fueled by fast-cycling stem cells. The number and identity of these dividing and migrating stem cells are maintained by a mosaic pattern at the base of the crypt. How the underlying regulatory scheme manages this dynamic stem cell niche is not entirely clear. We characterized Notch signaling in intestine stem cell niche by stimulating intestinal organoids with Notch ligands and inhibitors, and discovered that intestinal stem cells employ a positive feedback mechanism. ChIP-Seq data (H3K4me1 and H3K27ac) and motif analysis of mouse small intestinal cells identified a novel NICD binding site on a highly active enhancer region on the 2nd intron of Notch1, showing a direct Notch feedback loop that has not been reported in other systems. CRISPR/Cas9 editing system was applied to mutate Notch1 binding site in intestine organoid cells. The mutated binding site significantly altered the stem cell niche pattern. The forming efficiency and growth in CIRSPR/Cas9 mutated mouse and human organoids were significantly hindered consequently, impairing intestinal capability for regeneration. Dynamical system analysis and agent-based multiscale stochastic modeling suggest that the positive feedback enhances the robustness of Notch-mediated niche patterning. This study highlights the importance of feedback mechanisms in spatiotemporal control of the stem cell niche.

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F-3019

SINGLE-CELL RNA SEQUENCING REVEALS AGING-INDUCED QUIESCENCE IN NEURAL STEM CELLS OF THE SUBVENTRICULAR ZONE

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The adult rodent brain consists of two predominant neurogenic niches, the subgranular zone of the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles. Adult neurogenesis is regulated by a plethora of signals that are not only multidimensional – as they involve multiple cues– but they also vary dynamically with age. In the adult SVZ, neurogenesis is known to decline with aging however

the age-dependent mechanisms remain largely unexplored. Here, we performed single-cell RNA sequencing of neural stem cells from the aging brain to unravel age-associated changes. We identified that old neural stem cells are mostly in a quiescent state and they upregulate genes related to inflammation. Our analysis also revealed aging-specific regulatory networks that may be manipulated to increase the generation of adult-born neurons, which would counteract age-related behavioral decline.

F-3021

THE WOUND MICROENVIRONMENT REPROGRAMMES SCHWANN CELLS TO INVASIVE MESENCHYMAL-LIKE CELLS TO DRIVE PERIPHERAL NERVE REGENERATION

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Schwann cell dedifferentiation from a myelinating to a progenitor-like cell underlies the remarkable ability of peripheral nerves to regenerate following injury. However, the molecular identity of the differentiated and dedifferentiated states in vivo has been elusive. Here, we profiled Schwann cells acutely purified from both intact nerves and the wound and distal regions of severed nerves. Our analysis reveals novel facets of the dedifferentiation response, including acquisition of mesenchymal traits and a Myc module. Furthermore, wound and distal dedifferentiated Schwann cells constitute different populations, with wound cells displaying increased mesenchymal character induced by localised TGFb signalling. TGFb promotes invasion and, surprisingly, crosstalks with Eph signalling via N-cadherin to drive directional collective migration of the Schwann cells across the wound. Consistently, conditional Tgfbr2 deletion in Schwann cells resulted in stunted and misdirected reinnervation. Thus, the wound microenvironment is a key determinant of Schwann cell identity and promotes nerve repair through the integration of multiple concerted signals.

Funding Source: Medical Research Council

F-3023

IDENTIFICATION AND CHARACTERIZATION OF GENES RELATED WITH PARKINSON'S DISEASE

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Parkinson's disease (PD) is the most common neurodegenerative disease that characterized by gradual and progressive neuronal dysfunction. It caused by loss of dopaminergic neurons in substantia nigra pars compacta in midbrain. PD is classified into two types, familial PD and sporadic PD (SPD). Many researches have shown that several genomic mutations and risk factors are clearly involved in familial PD, however, few in sporadic PD. Here, we differentiated induced pluripotent stem cell (iPSC), generated from fibroblast of undiseased person (UND) and SPD patients, into midbrain dopaminergic neuron (mDA). We did mRNA-sequencing analysis with them, we found 20 genes that downregulated in SPD mDA compared to UND mDA. mRNA expression level is validated by quantitative real-time PCR in differentiated mDA. Two genes, X and Y, of 20 genes are selected. Protein expression levels of identified genes were less expressed in PD in-vitro model using the SH-SY5Y cell line which is consistent with the result of quantitative real-time PCR. It was reported that X is involved in WNT signaling. Y is known to adipocyte-specific chemokine receptor and reported that it is related to immune responses. These genes have not been studied yet in PD pathogenesis. Thus, our further study might contribute to understanding pathogenesis of sporadic PD.

F-3025

A HUMAN, IN VITRO MODEL OF STRETCH INJURY IN NEURONS TO STUDY GENE-TRAUMA INTERACTIONS

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Epidemiology suggests that genetic factors modulate the consequences of neurotrauma. Traumatic brain injury (TBI) increases the risk of several neurodegenerative disorders, including Parkinson's disease and Alzheimer's disease. Chronic traumatic encephalopathy (CTE) occurs only in individuals with a history of repeated mild TBI but most individuals with this history do not develop CTE. Human in vitro models have proven potential to shed new light on neurological disorders but they have not yet

been applied to CTE. This in vitro model applies a stretch insult to human induced pluripotent stem cell (iPSC)-derived neurons to induce neurotrauma pathology. It employs a 96 well format to minimize consumption of expensive cells and reagents. A silicone-bottomed 96 well plate is pressed down on to an array of vertical cylindrical posts to induce injury. The silicone stretches radially across the end of the post as the plate is lowered, stretching the cells cultured on it. The primary outcome measures are parameters measuring cell survival and morphology determined from fluorescent images of living cells. Increasing stretch caused a dose-dependent decrease in the neurite outgrowth/cell and cell survival in human iPSC-derived neurons (Cellular Dynamics Inc.). The pathognomic feature of CTE is its unusual spatial distribution. Tau pathology appears to radiate from small blood vessels and the depths of sulci in histology. These structures are strain concentrators. Therefore, tau pathology may begin at these structures shortly after trauma and radiate outwards into neighboring healthy cells over time. The in vitro stretch injury model has been adapted to study this phenomenon. When the posts in the stretching apparatus are not lubricated, the friction between them and the silicone prevents sliding. Therefore, the periphery of the membrane stretches as the plate is pressed on to the post but the central region touching the post does not. The goal of this work is to create co-cultures of traumatized and naive cells that can be used to study the propagation of pathology after trauma.

Funding Source: This work is supported by the National Institutes of Health (R21NS102579).

F-3031

HIGH-RESOLUTION FUNCTIONAL GENOMICS REVEALS CRITICAL VULNERABILITIES OF THE NURD COMPLEX FOR FETAL HEMOGLOBIN CONTROL

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Genetic disorders resulting from mutations of the adult beta-globin gene affect millions of people worldwide. These diseases manifest after birth once the fetal form of hemoglobin (HbF) is replaced by the adult form (HbA). An elevated level of HbF is without consequence in healthy individuals but conveys a major clinical benefit in patients with sickle cell disease and beta-thalassemia. A major goal is to develop a complete understanding of the gene regulatory mechanisms that act to silence fetal globin expression with the goal to reactive its expression in hemoglobinopathy patients. Tight control of gene expression involves an intricate network of DNA binding factors and chromatin readers, writers, and erasers acting within multiprotein nuclear complexes. The interfaces among protein partners comprise potential targets for rational drug development. The nucleosome remodeling and deacetylase (NuRD) complex is an epigenetic chromatin remodeler that has been implicated in HbF repression. In order to identify potential drug target domains (interfaces) within NuRD complex with maximal HbF de-repression and minimal cellular toxicity we have set out to dissect the assembly and activity of the NuRD complex in human umbilical cord blood-derived erythroid progenitor cells. We have performed CRISPR-Cas9 mediated saturating mutagenesis of the entire coding regions of all the human NuRD complex members in adult erythroid cells. This approach distinguished four classes of NuRD complex members: those required for HbF repression but dispensable for cellular fitness (such as MTA2, MBD2, HDAC2, and GATAD2A), those required for both HbF repression and cell fitness (such as CHD4), those only required for cell fitness (such as RBBP4), and many not essential for either HbF repression or cell fitness. We identified various conserved residues of NuRD members at which in-frame deletions result in elevated HbF levels, including some mapping to known domains and others to regions of unexplored functional significance. We are utilizing computational, structural, proteomics, and protein chemistry approaches to probe key interfaces that could subsequently serve as targets for small molecule development with the ultimate goal of therapeutic re-induction of HbF for the beta-hemoglobinopathies.

F-3033

PROMOTER METHYLATION REGULATED BY DNMT3A AND TET1 COMPETITION IN MOUSE EMBRYONIC STEM CELLS

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DNA methylation is a typical epigenetic marker, enabling stable but reversible gene repression. In mammalian cells, DNA methyltransferases DNMTs are responsible for modification from cytosine to 5-methylcytosine (5mC). Meanwhile, Tet dioxygenases can convert 5mC to 5-hydroxymethylcytosine (5hmC), facilitating DNA demethylation. However, the genome-wide cooperation and regulation of these two families

of proteins are largely unknown. Here we highlight Dnmt3a and Tet1 bind and function in a complementary and competitive pattern in mouse embryonic stem cells. The full-length Dnmt3a (Dnmt3a1) exhibits significant binding enrichment at distal promoters and the edges of large unmethylated regions (canyons), but exclusion from proximal promoters and canyons, precisely where Tet1 shows its highest genomic binding. Deletion of Tet1 increases Dnmt3a1 binding capacity at and around genes with the highest wild type Tet1 binding. However, deletion of Dnmt3a has no effect on Tet1 binding on chromatin, indicating Tet1 limits DNA methylation at least partially by protecting from Dnmt3a binding and setting up methylation boundaries. Furthermore, Dnmt3a and Tet1 impact histone modification and therefore regulate gene expression, in a reciprocal fashion. We propose that Dnmt3a and Tet1 corporately regulate the epigenome at specific genomic regions via their binding interactions.

F-3035

RELIABLE MODELING OF CORTICAL DEVELOPMENT AND MICROCEPHALY IN ROSETTES AND ORGANOIDS BY COMBINED PATHWAY INHIBITION

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Methods for specification of cortical fates from pluripotent stem cells (PSCs) are highly diverse, lack robust and direct readouts, and often yield heterogeneous progenitor populations enriched but not restricted to the cortical domain. Here we propose that enhanced Notch activation and efficient radial organization together serve as direct and robusts readout for successful specification towards homogeneous cortical progenitors. Both readouts can be achieved by combined dual-SMAD and WNT pathway inhibition. Neuroepithelial cells derived under combined inhibition exhibit enhanced Notch activation, remarkable radial organization (neural rosette formation), and homogenous and long-term cortical marker expression. Similarly-derived cerebral organoids exhibit Notch active and radially organized vesicles that homogeneously express cortical progenitor markers and retain proper cortical layer lamination. In contrast, inhibitor-free organoids exhibit substantially weaker Notch activation not necessarily overlapping with radial organization, suggesting heterogeneous specification. Interestingly, dual SMAD inhibition alone enhances Notch activation in non-radially organized regions primarily enriched for more caudal fates, while, WNT inhibition alone is sufficient to induce Notch activation and radial organization in organoids but not in monolayer cultures, emphasizing the unique ability of self-organized 3D structures to mimic correct cortical inductive signals. Microcephaly organoids harboring CRISPR mediated centrosomal defects exhibit smaller, impaired vesicles regardless of the differentiation paradigm, and show signs for cell cycle arrest. However, profound apoptosis is found preferentially when WNT is inhibited - reflecting selective damage to cortical regions. In support, caudal brain regions prevail when WNT is not inhibited - further confirming cortex specific cell death. Transcriptional profiling of microcephaly organoids reveals an intriguing set of cytosolic proteins whose expression is dysregulated only under combined inhibition. Thus, WNT and SMAD inhibition is indispensable for standardized modelling of corticogenesis in health and disease.

Funding Source: ISRAEL SCIENCE FOUNDATION

F-3037

TYPE I COLLAGEN BASED RECOMBINANT PROTEIN AS AN ADIPOCYTE DIFFERENTIATION INHIBITOR OF MESENCHYMAL STEM CELL

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Mesenchymal stem cell (MSC) has great potential for wide range of clinical application. It is very important to culture and provide MSC for injured tissue repair or treatment of diseases. In the ISSCR 2016, we showed that a type I collagen based recombinant protein (RCP) would promote cellular adhesion and proliferation of MSC only by direct instillation of this RCP solution into culture medium. The characteristic of the RCP is xeno-free, increased RGD (Arg-Gly-Asp) sequence and high uniformity of molecular weight. It is the material with biodegradability, biocompatibility, and cell adhesive property. In a process clarifying effects of the RCP, we found that it had not only the function of promoting cellular adhesion or proliferation, also had new function to inhibit adipocyte differentiation of MSC. The aim of this study is to make clear how does the RCP inhibit adipocyte differentiation of MSC. We used MSC derived from bone marrow or cartilage tissue, and media which were serum-free or containing serum. MSC was cultured in those media in which the RCP was contained or not. Then, MSC was cultured in each differentiation inducing culture media and dyed. The result showed that MSC differentiated to bone or cartilage tissue but not to adipocyte under the condition that the

RCP was contained in media. On the other hand, MSC differentiated to those three tissues in media which did not contain the RCP. We compared the differences in gene expression profile between the RCP was contained or not. Changes in adipogenesis related gene expression profile was monitored by RT-PCR (Human Adipogenesis PCR Array; QIAGEN). It was revealed that the RCP would inhibit adipocyte differentiation of MSC by depressing some adipogenesis related genes expression. Our findings indicate that adipocyte differentiation would be inhibited by adding the RCP into medium and that could be more effective the cell therapy using MSC.

F-3039

3D MODELING OF LUNG TISSUE TO INVESTIGATE TISSUE INJURY

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Bordetella pertussis is a gram-negative bacteria pathogen that causes the respiratory disease, Whooping Cough and has been associated with pulmonary hemorrhages and necrotizing bronchiolitis. In order to further understand the disease progression and pathology, we proposed the use of an engineered lung tissue as an in vitro model. Most cell behavior has been studied in the lab in a 2D monolayer culture. Due to the nature that cells in the body exist in a 3D environment, we propose the use of engineering a functional lung tissue in vitro to study the tissue damaged due to the Bp bacterium, as well as repair of tissue injury. We hypothesize that using decellularized lung extracellular scaffolds will support the engineering of functional lung tissue. Here, we developed a modified method to generate a 3D lung while maintaining the whole lung breathing mechanic. We decellularized the mouse lung tissue through the use of detergents through the trachea to minimize disrupting the lung tissue and retaining the ECM, while removing the cellular components. The lung scaffolds were recellularized using a bioreactor consisting of a peristaltic pump that periodically pushed media and differentiated cells into the suspended scaffold, recapitulating the expansion of lungs during breathing. In addition to our method of generating a scaffolding, we have developed a new differentiating method; mESC were differentiated in a 3D organoid matrigel culture in 150 ng/ml lung homogenate enriched EGM2 (Lonza) media supplement with 100 ng/ml Activin A for 4 days to drive toward definitive endoderm. To drive cells further toward lung, we cultured for an additional 6-8 days in media supplemented with 200 ng/ml Noggin for 6-8 days. Definitive endoderm was confirming with expression of SOX2, a foregut and proximal lung marker. Immunocytochemistry stain of the lung scaffolding verified that positive for TFF1, a lung marker; CD31, an endothelial cell marker, and ECadherin, a lung epithelial marker confirming a population of lung specific cells. Using this model as a platform, we will next study tissue repair during respiratory infection. We anticipate that this study will contribute important information about alternative lung tissue source for lung injury repair during pathogenic infection that may lead to faster recovery from whooping cough.

F-3041

ANTICIPATORY BIOMEDICAL ETHICS AND POLICY IMPLICATIONS FOR THE USE OF CRISPR TOGETHER WITH GENE DRIVE IN HUMANS

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Clustered regularly interspaced short palindromic repeats (CRISPR) genome editing has already reinvented the direction of genetic and stem cell research. For more complex diseases it allows scientists to simultaneously create multiple genetic changes to a single cell. Technologies for correcting multiple mutations in an in vivo system are already in development. On the surface, the advent and use of gene editing technologies is a powerful tool to reduce human suffering by eradicating complex disease that has a genetic etiology. Gene drives are CRISPR mediated alterations to genes that allow them to be passed on to subsequent populations at rates that approach 100% transmission. Therefore, from an anticipatory biomedical ethics perspective, it is possible to conceive gene drive being used with CRISPR to permanently ameliorate aberrant genes from wild-type populations containing mutations. However, there are also a number of possible side effects that could develop as the result of combining gene editing and gene drive technologies in an effort to eradicate complex diseases. We critically analyze the hypothesis that the combination of CRISPR and gene drive will have a deleterious effect on human populations from an ethical perspective by developing an anticipatory ethical analysis of the implications for the use of CRISPR together with gene drive in humans. We address the idea proposed by Kevin Esvelt that because gene drive genes would be so slow to spread in a population, there is time to create and release an "immunizing reversal drive" for the gene drive, thus rendering low risk in the immediate term. We couch our analysis in a practical ethics based on Robert Audi's moderate intuitionism. To complete our ethical analysis we overlay the properties of emergent ethics surrounding new technologies, namely George Lucas' "methodology of uncertainty". Finally, we outline a set of considerations for policy making surrounding of a CRISPR and gene drive system

in humans and what ethical considerations should be taken into account by policy makers based on this analysis.

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F-3043

ESOPHAGEAL REGENERATION WITH A CELL-SEEDED TISSUE ENGINEERED GRAFT

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Treatment of esophageal disease can necessitate resection and reconstruction of the esophagus. Current reconstruction approaches are limited to utilization of an autologous conduit such as stomach, small bowel, or colon. A tissue engineered construct providing esophageal replacement or regeneration for circumferential, full thickness defects would have significant clinical application. In the current study, we demonstrate that esophageal regeneration is feasible and reproducible in a large animal model using synthetic polyurethane electro-spun grafts seeded with autologous adipose-derived mesenchymal stem cells (aMSCs) and a disposable bioreactor. Animals underwent adipose tissue biopsy to harvest and expand autologous aMSCs for seeding on electro-spun polyurethane conduits in a bioreactor for 7 days. Confirmation of aMSC-based seeding was performed and anesthetized pigs underwent full thickness circumferential resection of the esophagus. Implantation of the cell seeded scaffold and esophageal stent deployment was performed, with scaffolds removed endoscopically after 3 weeks. Animals surviving up to 10 months showed durable tolerance to oral intake and gain weight. Progressive esophageal regeneration, mediated by a cellularized scaffold, was observed over the course of this study, as results from these animals showed gradual structural regeneration of endogenous esophageal tissue layers, including squamous esophageal mucosa, submucosa, and relevant smooth muscle with blood vessel formation. These results have implications for the esophagus as a regenerative organ, and for novel applications in human esophageal resection and reconstruction.

F-3047

MUTANT IPS CELLS DERIVED FROM PATIENTS WITH RALD SHOW SIGNIFICANCE OF KRAS FOR SELF-RENEWAL AND DIFFERENTIATION PROPENSITY

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KRAS is widely known as a proto-oncogene and has been reported to play essential roles in stemness maintenance in some types of stem cells, including cancer stem cells. However, the roles of KRAS in pluripotent stem cells (PSCs) are largely unknown. Recently, somatic gain-of-function mutations of KRAS or NRAS in hematopoietic stem cells have been reported to cause RAS-associated autoimmune lymphoproliferative syndrome-like disease (RALD). Here, we investigated the roles of KRAS on stemness maintenance in the context of human PSCs using isogenic KRAS mutant (G13C/WT) and wild-type (WT/WT) induced PSCs (iPSCs), generated from the same RALD patients with the somatic KRAS mutation. Using the isogenic iPSC lines from two patients, we revealed that G13C/WT iPSCs displayed self-renewal and differentiation characteristics distinct from those of WT/WT iPSCs: expression of stemness markers, including POU5F1 and NANOG, was maintained at high levels in G13C/WT iPSCs under bFGF depletion; neuronal differentiation was clearly blunted from G13C/WT iPSCs. In addition, we generated wild-type (WTed/WT) and heterozygous knockout (Δed/WT) iPSCs from the same G13C/WT clone using gene-editing techniques. As expected, the G13C/WT-specific phenotypes were normalized in WTed/WT iPSCs. Interestingly, Δed/WT iPSCs showed lower potential to maintain undifferentiation status under bFGF depletion, with a higher tendency to differentiate into neuronal lineage than WTed/WT iPSCs. Biochemical analysis indicated hyper-activation of KRAS and subsequent increased phosphorylation levels of ERK in G13C/WT iPSCs. Pharmacological studies using specific kinase inhibitors demonstrated that the features compatible with enhanced stemness maintenance were canceled in mutant cells by pan-RAF and MEK inhibitors, but not

by PI3K inhibitors. In addition, neuronal differentiation was improved in G13C/WT iPSCs with the MEK inhibitor treatment. These observations suggested that the KRAS–ERK pathway plays more critical roles than the KRAS–PI3K pathway in G13C/WT iPSCs. Collectively, the analyses on the isogenic and genome-edited iPSCs from the RALD patients revealed the crucial roles of the KRAS–ERK signaling on the stemness maintenance, having a strong impact on self-renewal and differentiation propensity in human iPSCs.

F-3051

THREE WAYS TO ACTIVATE THE SAME PATHWAY: COMPARISON OF THE CANONICAL WNT ACTIVATION MODES DURING HUMAN ESC DIFFERENTIATION IN VITRO.

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GSK-3 inhibitor CHIR99021 has been widely used to mimic the activation of the canonical Wnt pathway as part of in vitro differentiation of human pluripotent stem cells into various lineages and is considered to be a "better alternative" for Wnt3 ligands. However, no unbiased transcriptome-wide comparison of its effects to those of the recombinant Wnt3 protein has been performed. Here I systematically analyze and compare the temporal transcriptional response of human embryonic stem cells to three modes of Wnt pathway activation: GSK3 inhibition by CHIR, treatment with the recombinant Wnt3a protein and the overexpression of the constitutively active beta-catenin mutant. Expectedly, the analysis highlights the quick activation of known primitive streak/mesoderm-associated genes between 8 and 24 hours of treatment in all 3 conditions, in accordance with the major role of Wnt pathway in the primary gastrulation. However, starting from 24 hours of treatment, CHIR and b-catenin, but not Wnt3a protein, show steady increase in expression of genes associated with paraxial/somitic mesoderm (including HOX gene cluster) and the neural tube/neural crest formation, pointing towards the known role of Wnt pathway in so-called secondary gastrulation (neural tube formation). On the other hand, Wnt3a protein at this point causes a strong upregulation of definitive endoderm markers (e.g. SOX17) and over time displays higher heterogeneity of the cell fates in the differentiating culture. Signaling pathway analysis indicates that up to 24 hours of stimulation, the observed effects of CHIR are apparently conferred mainly through the activation of beta-catenin-mediated transcription, while there is a transcriptional expansion of 3 major endogenous signaling networks (FGF, non-canonical Wnt and BMP) that could explain observed tendencies in cell fate choices at later time points. In conclusion, this study highlights the consequences of activating canonical Wnt pathway at different levels and uncovers the candidate endogenous pathways that could be manipulated in attempts to deliver more precise steering of early mesodermal differentiation in vitro.

F-3053

REGULATION OF DIFFERENTIATION BY RHOA IN MOUSE EMBRYONIC STEM CELLS

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RhoGTPases play important roles in the regulation of actin cytoskeleton that in turn is crucial for cell migration, cell adhesion, cell proliferation and other cellular activities. RhoA, a well characterized RhoGTPase, induces the formation of actin stress fibers in fibroblasts and epithelial cells. Previous study has shown that knocking out RhoA in mouse leads to embryonic lethality, suggesting the importance of RhoA during embryonic development. Although the roles of RhoA in late developmental stages have been reported, little is known about the function of RhoA in early embryonic development. This study aims to investigate the role of RhoA in the regulation of differentiation and lineage specification in mouse embryonic stem cells (mESCs). Immunoblot analysis reveals that protein levels of RhoA in mESCs increase gradually during differentiation. The expression of constitutively active RhoA in mESCs directs differentiation towards endodermal and mesodermal lineages, while inhibiting differentiation towards ectodermal lineage.

F-3055

ENHANCED THERAPEUTIC AND LONG-TERM DYNAMIC VASCULARIZATION EFFECTS OF HUMAN PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS ENCAPSULATED IN A NANOMATRIX GEL

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BACKGROUND: Human pluripotent stem cell (hPSC)-derived endothelial cells (ECs) have limited clinical utility due to undefined components in the differentiation system and poor cell survival in vivo. Here, we aimed

to develop a fully defined and clinically compatible system to differentiate hPSCs into ECs. Further, we aimed to enhance cell survival, vessel-formation, and therapeutic potential by encapsulating hPSC-ECs with a peptide amphiphile (PA) nanomatrix gel. METHODS: We developed a fully defined system free of xenogeneic components to differentiate hPSCs into ECs by treatment including a GSK3β inhibitor and a Notch ligand, and sorting with CDH5. We constructed an extracellular matrix-mimicking PA nanomatrix gel (PA-RGDS) by incorporating the cell adhesive ligand Arg-Gly-Asp-Ser (RGDS) and a matrix metalloproteinase-2 degradable sequence. We then evaluated whether the encapsulation of hPSC-CDH5+ cells in PA-RGDS could enhance long-term cell survival and vascular regenerative effects in a hindlimb ischemia model using Laser Doppler perfusion imaging, bioluminescence imaging, real-time RT-PCR, and histological analysis. RESULTS: The resultant hPSC-CDH5+ cells showed genuine EC characteristics, pro-angiogenic activities, and vascular regenerative effects. When encapsulated in a peptide amphiphile (PA) nanomatrix gel and implanted in a mouse hindlimb ischemia model, they induced better therapeutic results than bare hPSC-ECs and showed unprecedented long-term survival in vivo (> 10 months). Surprisingly, the engrafted hPSC-ECs demonstrated previously unknown sustained and dynamic vessel-forming behavior: initial perivascular concentration, a guiding role for new vessel formation, and progressive incorporation into the vessels over 10 months. CONCLUSION: We generated highly enriched hPSC-ECs via a clinically compatible system. Further, this study demonstrated that a biocompatible PA-RGDS nanomatrix gel substantially improved long-term survival of hPSC-ECs in an ischemic environment and improved neovascularization effects of hPSC-ECs via prolonged and unique angiogenic and vessel-forming properties. This PA-RGDS-mediated transplantation of hPSC-ECs can serve as a novel platform for cell-based therapy and investigation of long-term behavior of hPSC-ECs.

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F-3057

MAKING MULTIPLE THERAPEUTIC CELL PRODUCTS FROM A CGMP-COMPLIANT IPSC LINE

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Induced pluripotent stem cell (iPSC) can differentiate into multiple phenotypes, and indeed multiple protocols for such differentiation have been established. Although the efficiency of different protocols is variable, most protocols work with most lines with some tweaking. To confirm this hypothesis and to make a clinically compliant line available for independent evaluation, we have taken the current Good Manufacture Practice (cGMP)-compliant iPSC line developed by the NIH, and used it to develop cellular products that are currently being considered for therapy. We focused on retinal cells, MSC, NSC, dopaminergic neurons and astrocytes. We obtained a WCB from the NIH and prepared a stock at passage 20. After testing quality we used it to generate multiple products using standard protocols. To confirm that these protocols could also be used for other iPSC lines we obtained a second clinically compliant line and tested the reproducibility of our methodology. Our results confirmed that the same protocols could be used with minimal modifications with multiple qualified lines. We believe that our demonstration that multiple products can be made from the same WCB bank and the same protocols can be used with multiple lines offers a path to a cost effective strategy for developing cellular products.

F-3061

TRUNCATING MUTATIONS IN THE POLYCOMB FACTOR ADDITIONAL SEX COMBS-LIKE 1 IMPAIR HUMAN NEURAL CREST CELL DIFFERENTIATION

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Additional Sex Combs-like 1 (ASXL1) is involved in Polycomb-dependent gene regulation via two antagonistic mechanisms: enhancing H3K27me3 deposition by recruitment of PRC2, or promoting removal of H2AK119 and subsequently H3K27me3 via activation of the BRCA1 Associated Protein 1 (BAP1). The functional importance of these mechanisms for embryonic development however remains unclear. It has recently been reported that heterozygous mutations in ASXL1 lead to Bohring-Opitz-Syndrome (BOS), a developmental disorder. We found that BOS-patient-derived iPSCs, as well as hESC bearing similar ASXL1 mutations, express truncated, dominantly acting

ASXL1 proteins (mutASXL1). Strikingly, differentiation to migrating neural crest-like cells was strongly impaired in mutASXL1-expressing hESCs. Transcriptional downregulation of several neural crest fate determinants, most prominently of ZIC1, was underlying the decreased developmental capacity. Interestingly, we observed a trend of increased presence of H3K27me3 at both ZIC1 and the neighboring gene ZIC4, which is also implicated in neural crest development. We believe our observations can explain the development of several neural crest- related symptoms in BOS, and are a first step towards understanding the role of (truncated) ASXL1 in Polycomb/BAP1-mediated regulation of development.

F-3063

DEVELOPING AN IPSC-DERIVED MODEL SYSTEM TO STUDY THE REGULATION OF SNCA EXPRESSION IN THE CONTEXT OF THE HETEROGENEITY IN SYNUCLEINOPATHIES.

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Synucleinopathies are a group of neurodegenerative disorders that share a common pathological hallmark of intracellular aggregates, mainly composed of α-synuclein. This study has focused on the most common synucleinopathies, Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB). Both pathologies show intracellular α-synuclein aggregates, however, they have distinct characteristics with respect to the cell type containing the aggregates and their anatomical distribution in the brain. While the dopaminergic circuit is predominantly affected in PD, the neurons first affected in the early stage of DLB are cholinergic. The expression levels of the SNCA gene are important for the development of synucleinopathies, however, the molecular mechanisms regulating SNCA expression and their contribution to the phenotypic heterogeneity of synucleinopathies are largely unknown. To study the differential regulation of SNCA expression in the context of PD compared to DLB, we have established an isogenic induced Pluripotent Stem Cell (iPSC)-derived system. iPSCs from a normal subject and a patient with SNCA-triplication (SNCA-tri) were differentiated into dopaminergic and cholinergic neurons to model PD and DLB, respectively. The isogenic lines were characterized on both phenotypic and molecular levels throughout the stages of differentiation. The iPSC-derived dopaminergic and cholinergic neurons showed cell-specific vulnerability to PD related phenotypes: mitochondria dysfunction, apoptosis, autophagy and aging. On a molecular level, we observed a specific profile for each cell type of conserved miRNAs targeting the SNCA-3'UTR. The mature cholinergic and dopaminergic neurons exhibit differential expression of miR7-5p, miR153-3p, and miR223-3p. The SNCA-tri derived lines showed similar trends for the studied miRNAs. However, the comparison between the control and the SNCA-tri derived neurons showed an increase in miR223-3p and a 10x decrease in the miR7-5p. Furthermore, we detected a cell-specific profile for SNCA alternative transcript variants in iPSC-derived dopaminergic and cholinergic neurons. Collectively, these results suggest that common and distinct regulatory mechanisms of SNCA gene expression might contribute, at least in part, to the etiology of synucleinopathies.

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F-3065

INVESTIGATING LOW-GRADE AND HIGH-GRADE GLIOMAGENESIS USING BRAIN TUMOUR-DERIVED IPSCS

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Gliomas are incurable brain cancers. They can arise de novo in their most malignant form (glioblastoma multiforme, GBM) or as low-grade tumours that often transform into GBM. Prognosis is very poor despite treatment and the development of novel therapies is hindered by a poor understanding of the underlying biology of gliomagenesis, treatment resistance and recurrence. New approaches are urgently needed to address these challenges. To this end, we derived 3 iPSC models from primary brain tumour tissue;

representing both low- and high-grade gliomas. Deep whole genome sequencing indicates preservation of mutational variants in the glioma iPSC models compared with their parental tumours. Neuronal differentiation in 2-dimensional (adherent) and 3-dimensional (organoid) culture revealed that the high-grade glioma iPSCs in particular fail to terminally differentiate. This 'differentiation block' phenotype was characterised by a high proportion of SOX1/SOX2/KI67 positive neural rosettes, and consistently, in vivo orthotopic implantation of neural cancer stem cells from our iPSC lines led to the formation of GBM-like xenograft tumours. Encouraged by the finding that our glioma iPSCs show the expected tumorigenic capacity, we carried out comparative transcriptional profiling using mRNA-seq. Notably, gene set enrichment analysis has started to reveal underlying regulatory networks that may be responsible for the phenotypic differences between the control, low- and high-grade glioma iPSC lines. Accordingly, our iPSC models may shed new light onto the processes of malignant transformation and cancer stem cell self-renewal, and ultimately they may inform precision medicine approaches targeting glioma progression and recurrence.

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F-3067

HOW AN EMBRYO KNOWS ITS BOUNDARIES: TIMING AND SPECIFICITY OF EPHRIN-B1-MEDIATED CELL SEGREGATION IN CRANIOFRONTONASAL SYNDROME

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Congenital craniofacial disorders are among the most common birth defects, but the underlying cellular mechanisms of many of these syndromes remain incompletely understood. Craniofrontonasal syndrome (CFNS) is an X-linked disorder caused by mutations in EFNB1, which encodes EPHRIN-B1, a member of the Eph/ephrin family of signaling molecules. Unlike most X-linked conditions, heterozygous females are more severely affected than hemizygous males, a unique inheritance pattern caused by mosaicism for EPHRIN-B1 expression after random X inactivation in heterozygous females. In Efnb1+/- mice, mosaicism leads to active segregation between Ephrin-B1 expressing and non-expressing cells in the neural plate neuroepithelium. Aberrant Ephrin-B1 expression boundaries are also present in craniofacial structures later in development, and often correlate with dysmorphogenesis. Using an hiPSC-derived neuroepithelial cell model system for CFNS, we show that mosaicism for EPHRIN-B1 expression drives aberrant cell segregation in human neuroepithelial cells, providing evidence that this abnormal cellular behavior is relevant to disease pathogenesis. To determine whether aberrant boundaries in craniofacial development are created only by early neuroectodermal cell segregation, or whether cell segregation is ongoing throughout development, we have generated Ephrin-B1 mosaicism in different cell populations at different developmental times using tissue-specific Cre recombinase-expressing mouse lines. Pinpointing the cell types capable of undergoing self-organization by Ephrin-B1 mosaicism will allow us to determine the cellular etiology of CFNS and the relative contributions of different cell types to disease pathology. This will also provide a greater understanding of the Eph/ephrin signaling-mediated coordination required to drive craniofacial development and morphogenesis.

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F-3069

POPULATION-SCALE ANALYSIS OF CELLULAR GENOTYPE-PHENOTYPE RELATIONSHIPS BY SINGLE-CELL RNA SEQUENCING

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The high polygenicity of neuropsychiatric disorders – which are shaped by hundreds to thousands of common and rare variants, and by different combinations of risk variants in each individual – presents formidable challenges for biology. Common variants offer many potential insights about quantitative, cell-type-specific perturbations of genes' expression levels, but the numbers of common variants and cell types to explore are both vast. Generalizations from disease modeling studies that use a small number of cell lines have often met limited success in downstream applications, including clinical trials. This may in part be because such studies (i) are not appropriately powered to provide tractable signals that connect disease associated genetic variants to their biological effect; (ii) do not appreciate the effects of diverse genetic backgrounds; and (iii) do not adequately address technical and experimental sources of noise. We have been working to develop a novel strategy for population-scale cellular studies: pooling cell lines from ever-increasing numbers of individuals, then using sequencing-based strategies for deconvoluting cells' identities and representations as

part of a phenotypic readout. Pooling lines from many donors and growing them together as an ensemble isolates genetic analysis from the well-to-well variance that often confounds biological experiments, offering an internally controlled system in which genetic influences on phenotypes may be most clearly appreciated. The scalability and resource efficiency of our experimental system allows us to quickly and efficiently pursue several goals: (i) identify expression QTLs in a variety of in vitro derived cell types; (ii) identify expression response-QTLs under a variety of perturbed conditions; and (iii) reveal the genetic architecture of many phenotypes for which cellular pools can be sorted or selected. In all cases, a central goal is to assess genetic correlation with human phenotypes from genetic studies so that we can increase focus on cellular phenotypes that genetic analyses indicate may be relevant to pathophysiological processes.

F-3071

THE ROLE OF FGF2 IN THE REPROGRAMMING OF EPIDERMAL KERATINOCYTES TOWARDS NEURAL CREST FATE

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Neural crest cells (NC) play a central role in the formation of the peripheral nervous system, the craniofacial skeleton and pigmentation of the skin during embryonic development. Due to their broad differentiation potential these cells have been a focal point of research for disease therapeutics via cell transplantation. However, an easily accessible autologous cells source for therapeutic cell transplantation remains one of the main challenges facing the field. Recently our lab discovered that human epidermal inter-follicular keratinocytes (KC) can turn into neural crest (NC) stem cells without ectopic expression of transcription factors or reprogramming to the pluripotent state. Here we studied the role of FGF2 in this novel phenomenon. We identified two pathways to be critical for reprogramming leading to expression of the NC-specific transcription factors including SOX10, Pax3, FoxD3 as well as intermediate filament gene Nestin. Most notably, KC-NC could be coaxed to differentiate into peripheral neurons, Schwann cells, melanocytes and smooth muscle cells as shown by molecular as well as functional assays. In conclusion, we provide mechanistic insight into the process of keratinocyte reprogramming to neural crest stem cells. This work represents a paradigm shift in stem cell biology as it demonstrates the unusual plasticity of human adult KC that can turn into many different cells types without genetic modification or reprograming to the pluripotent state. The dearth of cell sources for treatment of neurogenic disorders, combined with the accessibility and growth potential of human epidermal cells suggest that the proposed work could have tremendous implications for the use of cell therapy for treatment of many debilitating diseases.

F-3073

STANDARDIZED GENERATION OF PATIENT-SPECIFIC AND GENE-CORRECTED IPSC LINES FOR DISEASE MODELING AND DRUG SCREENING

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The use of pluripotent stem cell-derived cells for disease modeling, drug screening and regenerative medicine is an exciting area of activity in health research. Induced pluripotent stem cell (iPSC) technology enables the generation of patient-specific pluripotent cell lines, making personalized medicine approaches possible. Furthermore, recent advances in genome editing technologies enable the efficient creation of genetically corrected iPSC lines. At CCRM, we have established an iPSC production facility focused on generating high quality pluripotent cell lines from patient samples for academic researchers and clinicians. Fully operational for four years, CCRM has delivered over 100 patient iPSC lines that are being used for disease modelling, and in drug screening initiatives, at Institutes across Canada. Specializing in non-integrative reprogramming technologies, we have developed SOPs to reprogram many common cell types in feeder-free conditions, including dermal fibroblasts, bone marrow stromal cells, cord and peripheral blood, and endothelial cells. We are working with The Hospital for Sick Children in Toronto and Cystic Fibrosis Canada on their program to create a community resource of 100 patient-specific iPSC lines to be used in drug screening and personalized medicine projects. Finally, CCRM has established a gene editing workflow that uses CRISPR/Cas9 technology to generate genetically modified iPSCs, including the induction of mutations of interest in control cells and the correction of genetic mutations in patient lines. Access

to patient-derived iPSCs and the associated isogenic controls allow for the discovery of novel disease-associated phenotypes and therapeutic targets for specific patient subpopulations.

F-3075

REPROGRAMMING ENRICHES FOR SOMATIC CELL CLONES WITH SMALL SCALE MUTATIONS IN CANCER-RELATED GENES

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Recent studies demonstrated that the observed high mutational load in induced pluripotent stem cells (iPSCs) is largely derived from their parental cells, but it is not known whether reprogramming may enrich for specific mutations that pre-exist in the mosaic parental cell population. We have derived 30 human iPSC lines from endothelial cells of neonatal and aged individuals under comparable conditions. High accuracy exome and amplicon sequencing showed that all analyzed 'true' SNPs and INDELs pre-existed in their parental cells. Importantly, we provide first evidence that individual mutations present in small subpopulations of parental cells become highly enriched among iPSC clones during reprogramming. Most of the genes affected by such mutations that became enriched are involved in the control of cell cycling, cell death or in stress responses. Disconcertingly, the majority of them is known to be cancer-related. Moreover, iPSCs from elderly patients show an increased number of such enriched mutations. These findings question the significance of reprogramming-induced de novo mutations in iPSCs and support the assumption that iPSCs from elderly patients are of lower biological quality. Reprogramming-associated selection for individual potentially pathogenic mutations that have been acquired during lifetime may impact the clinical value of patient-derived iPSCs.

F-3077

MESENCHYMAL STEM CELL LIFESPAN EXTENSION VIA INHIBITION OF MTOR SIGNALING CORRELATES WITH EXPRESSION OF SPECIFIC SENESCENCE MARKERS

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Replicative senescence of human mesenchymal stem cells (MSCs) limits the therapeutic potential for tissue engineering and regenerative medicine. Several key molecular players involved in the establishment of cellular senescence have been identified, among them is the mTOR signaling pathway. Persistent activation of mTOR signaling has been shown to drive MSCs senescence and, importantly, dampening mTOR activity with rapamycin, a specific mTOR inhibitor, was shown to maintain self-renewal of these cells during long-term culture expansion. However, the mechanisms by which mTOR inhibitors favors stem cell lifespan are still poorly understood and it is unknown whether individual differences in the response to mTOR inhibitors exist. To further explore these questions, we continuously blocked mTOR activity with rapamycin during in vitro expansion of MCSs derived from bone marrow of 5 healthy male young donors and analyzed the number of cell population doublings until growth arrest of each sample in both conditions: rapamycin-treated and untreated condition. The timing when untreated cells entered growth arrest was herein referred as "deviation" passage. We observed that MSCs from different donors presented variable responses to rapamycin: whereas rapamycin extended dramatically the lifespan of 1 sample (278% increase in lifespan), it had only subtle effects on serial expansion of 3 samples (126%-150% increase in lifespan), and no impact on another sample. This effect on the responding sample was mediated by the continuous inhibition of mTOR, as cells quickly undergo replicative senescence upon rapamycin removal. Interestingly, the lower the fold of the senescence marker P16 protein expression between treated and untreated cells at deviation, the greater the number of population doublings under rapamycin treatment. In addition, rapamycin significantly reduced secretion of senescence-associated cytokines IL6 and IL8. Overall, although there is variability in rapamycin-induced lifespan, a clear correlation between fold expression of P16 and extended lifespan with rapamycin exists and an effect of rapamycin in reducing ILs production was observed in MSCs, effects that may contribute to the retention of the proliferative capacity of rapamycin-treated cells.

F-3079

UBIQUITIN C DECREMENT PLAYS A PIVOTAL ROLE IN REPLICATIVE SENESCENCE OF HUMAN BONE MARROW MESENCHYMAL STROMAL CELLS

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Human bone marrow-mesenchymal stromal cells (hBM-MSCs) undergo cellular senescence during in vitro culture. In this study, we defined this replicative senescence as impaired proliferation, deterioration in representative cell characteristics, accumulated DNA damage and decreased telomerase activity with or without genomic abnormalities. Gene expression analyses demonstrated that the UBC gene located in the center of molecular networks associated with the replicative senescence. The UBC gene expression gradually decreased during passaging along with the reduction in series of molecules including hub genes; CDK1, CCNA2, MCM10, E2F1, BRCA1, HIST1H1A and HIST1H3B. UBC knockdown in hBM-MSCs induced impaired proliferation in dose dependent manner and showed replicative senescence-like phenomenon. Gene expression changes after UBC knockdown were similar to late passage hBM-MSCs. In addition, UBC worked in higher-order through regulating the hub genes controlling cell cycle and proliferation. These results indicate that the decrement of UBC expression plays a pivotal role in replicative senescence of hBM-MSCs.

Funding Source: This research was supported by a grant (14172MFDS974) from Ministry of Food and Drug Safety in 2016 and a grant of the Korea Health Technology R&D Project by the Ministry of Health & Welfare, Republic of Korea (grant number: HI15C3076).

F-3081

IMMUNOMODULATORY EFFECTS OF TONSIL-DERIVED MESENCHYMAL STEM CELLS CONDITIONED MEDIUM TREATED WITH LOW-LEVEL LIGHT IN AN ALLERGIC RHINITIS MOUSE MODEL

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We investigated whether low-level light irradiation prior to treatment of Tonsil-derived mesenchymal stem cells conditioned medium (TMSC-CM-LLLT) in an experimental allergic rhinitis (AR) model exhibited immunosuppression to healing allergic rhinitis. Tonsil-derived mesenchymal stem cells (TMSC), which can differentiate into several lineages, exhibit immune suppressive effects in allogeneic transplantation. These unique properties make TMSC of great interest for clinical applications in immunosuppression. However, there is no report that evaluates the in vivo immune-modulating effect of Tonsil-derived mesenchymal stem cells conditioned medium (TMSC-CM) in an experimental AR model. The effect of TMSCwas evaluated in 24 BALB/c mice that were randomly divided into four groups (negative control group, positive control group, TMSC group, TMSC-LLLT group). TMSC-(CM) or TMSC-(CM)-LLLT were administered intravenously to ovalbumin (OVA) sensitized mice on days 0 to 14, and subsequent OVA challenge was conducted daily from days 21 to 27. The immunomodulatory effects of TMSC were evaluated by nasal symptoms, eosinophil infiltration, serum total, OVA-specific immunoglobulin E (IgE), IgG1, IgG2a, IL cytokine profile, and neutrophils. In addition, they expressed immono-modulatory factors of MSCs, such as transforming growth factor beta (TGF-β), hepatocyte growth factor (HGF), and prostaglandin E2 (PGE2), but not that of IL-10. TMSC-CM-LLLT significantly reduced allergic symptoms and inhibited eosinophilic inflammation in the nasal mucosa. TMSC-CM-LLLT significantly decreased the total IgE, the OVA-specific IgE, IgG1, and IgG2a level in the AR mouse model. TMSC-CM-LLLT inhibited IL-4, IL-5, IL-6, IL-10, IL-17, and IFN-γ production from OVA-incubated splenocytes. In addition, the TMSC-CM-LLLT group had more inhibition of allergic inflammation than did the control group. Therefore, it is suggested that TMSC-CM-LLLT may be used as an alternative strategy to adult MSCs in the treatment of allergic rhinitis.

F-3083

GENERATING MESENCHYMAL STEM CELLS IN 3D FROM HUMAN EMBRYONIC STEM CELL SPHEROIDS

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Mesenchymal stem cells (MSC) derived from human embryonic stem cells (hESC), named EMSC here, have been found efficacious in animal models of autoimmune, inflammatory, and degenerative diseases. However, all the EMSC derivation methods reported so far are in two-dimensional (2D) culture systems, which are of low efficiency and high cost, difficult for large-scale production for research and therapeutic applications. We established a 3D system that allowed differentiation of hESC spheroids into MSC spheroids (EMSCSp) following treatment with BMP4 and A8301 for 5 days and subsequent culture in a MSC medium for about 15 days. All the procedures were conducted in one vessel without intermediate passaging. EMSCsp cells were efficiently derived from hESC spheroids within 20 days in the 3D culture system, which could be scaled up from a small culture vessel to a 100-ml plastic bag. EMSCSp could further differentiate into spheroids of chondrocytes or adipocytes. EMSCSp could also reattach and form a 2D-monolayer culture (EMSCSp-ML). Compared to EMSC differentiated in monolayer, EMSCSp-ML had faster proliferation and higher yield, and developed less apoptosis and slower senescence. EMSCSp-ML also retained immune-modulatory effects in vitro and therapeutic effects on two mouse models of colitis. The 3D method provides a simple and economic system for large-scale production of EMSC as an unlimited source of the therapeutically promising cells.

Funding Source: This work was supported by University of Macau Research Committee funds SRG #2014-00008-FHS and MYRG #2015-00169-FHS and 2016-00070-FHS, and Macau Science and Technology Development Fund (FDCT) #128-2014-A3 and 028/2015/A1 to R.X.

FRIDAY, JUNE 16, 2017

LATE BREAKING POSTER SESSION IIII-EVEN 19:00 – 20:00

F-3002

EFFECTS OF BMP-2 MEDIATED OSTEOGENIC DIFFERENTIATION IN BONE MARROW STEM CELLS OF FIBROUS DYSPLASIA

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Fibrous dysplasia (FD) is a rare skeletal disease with somatic mutation in the GNAS1, located on chromosome 20q13.2. In FD patients, stem cells in affected bone is affected by the mutation, resulting in weak bone formation with proliferation of immature osteogenic cells. Lesions may cause abnormal bone growth, deformity, or fracture. However, the current care of fibrous dysplasia is surgical removal of excess bulk lesion, which causes facial disfigurement. This approach is prone to face the recurrence of FD lesion. The purpose of this study was to evaluate the effects of BMP-2 in bone-grafting procedures of fibrous dysplasia. Normal bone marrow stem cells from maxilla (BMSCs) was compared to bone marrow stem cells from maxilla of fibrous dysplasia patients (FD-BMSCs). FD-BMSCs were analyzed with gene sequencing for GNAS1 mutations. Proliferation was determined with colony-forming assay, CCK-8, and BrdU staining. OCT4 and NANOG were analyzed with flow cytometry and immunocytochemistry. Osteogenic differentiation of both BMSCs and FD-BMSCs was performed with ALP activity and alizarin red S staining. Gene expressions for osteogenic markers, osteocalcin and type 1 collagen, were determined with RT-PCR. From transplantation on immunodeficient nude mice, the bone formation of both BMSCs and FD-BMSCs were observed. FD-BMSCs have less cell viability than BMSCs. Also, OCT4 and NANOG on BMSCs were stained more than FD-BMSCs. By comparing the osteogenic differentiation, FD-BMSCs showed low calcium deposits and ALP activity. However, there were overcome on low levels of osteogenic differentiation in FD-BMSCs with BMP-2 treatment. The transplanted FD-BMSCs also formed low levels of bone formation on the mouse. The results present that FD-BMSCs have low proliferation rate and osteogenic differentiation. Overall, BMP-2 treatment helps the increase of osteogenic differentiation on

FD-BMSCs. This suggests that BMP-2 grafts should be a good candidate for surgical treatment of fibrous dysplasia patients.

F-3004

SINGLE-CELL TRANSCRIPTOME PROFILING UNCOVERS AN ECTOPIC INTERCELLULAR SIGNALING CENTER IN HIPPO DEFICIENT HEARTS

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Epicardium is the outmost layer of heart and serves as essential progenitors of non-cardiomyocytes lineage. Epicardium is also a critical signaling center regulating myocardium growth and coronary vessel development. Although the lineage contribution of epicardium is known, the mechanisms controlling epicardial lineage choices are poorly understood. The Hippo signaling pathway, a conserved kinase cascade for organ size control is known to play a pivotal role in cell proliferation and fate determination. To study Hippo signaling in epicardium, we inactivated Hippo signaling kinase Lats1/2 in Wt1 epicardial lineage at E11.5. Lats mutant hearts are embryonic lethal at E15.5. To gain molecular mechanism, we performed Drop-seq to profile single cell transcriptome. Transcriptome signature of 18166 cells from wild type and Lats1/2 CKO hearts at E13.5 and E14.5 were profiled. A unique cluster of predominantly mutant cells resides in subepicardium which are arrested during the differentiation of epicardial cells to cardiac fibroblasts in Lats1/2 CKO hearts. This cluster of cells is featured with the expression of intercellular signaling factors which are known to regulate vessel development. Linage tracing experiment showed epicardial cells in Lats kinase deficient heart preferentially differentiate into endothelial cells versus fibroblasts and smooth muscle cells. Although with increased number of endothelial cells, the pattern of coronary vessel is disorganized with reduced branching points. Monocle pseudotime estimation algorithm delineated the differentiation process from epicardium to fibroblast. It showed that the unique cluster uncovered in Lats deficient heart formed an unexpected branch and was unable to complete the differentiation process. The genes which led to this differentiation defect and intercellular signaling factor expression exhibits enriched TEAD motif, suggesting the activation of Hippo down-stream effector Yap. Taken together, Hippo kinase Lats is essential for the cellular contribution and signaling secretion from epicardium during heart development. It suppresses endothelial cell specification from epicardium and facilitates differentiation from epicardium to cardiac fibroblasts.

F-3006

ZFP521 REGULATES HEMATOPOIETIC STEM CELL FUNCTION AND FACILITATES MLL-AF9 LEUKEMOGENESIS

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The concept that tumor-initiating cells can co-opt the self-renewal program of endogenous stem cells as a means of enforcing their unlimited proliferative potential is widely accepted, yet identification of specific factors that regulate self-renewal of normal and cancer stem cells remains limited. Using a comparative transcriptomic approach, we identified ZNF521 and Zfp521 as a conserved hematopoietic stem cell (HSC)-enriched transcription factor in human and murine hematopoiesis, respectively, whose role in HSCs remains to be fully elucidated. Competitive serial transplantation assays using Zfp521-deficient mice revealed that ZFP521 regulates HSC self-renewal and differentiation. In contrast, enforced ectopic expression of ZFP521 in HSCs led to a robust maintenance of progenitor activity in vitro. Transcriptional analyses of human acute myeloid leukemia (AML) patient samples revealed that ZNF521 is highly and specifically up-regulated in AMLs with MLL translocations. In vivo experiments showed that while ZFP521 was not strictly required for leukemogenesis, increased survival was observed following primary and secondary transplantation in the absence of ZFP521 using an MLL-AF9-mediated murine leukemia model. Furthermore, knockdown of ZNF521 led to reduced proliferation in human leukemia cell lines possessing MLL-AF9 translocations. Taken together, these results identify ZNF521/ZFP521 as a critical regulator of HSC function, which facilitates MLL-AF9-mediated leukemic disease in mice.

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F-3008

INCREASED SRF/MKL1 BINDING AT TARGET GENOMIC LOCI AUGMENTS MEGAKARYOCYTIC MATURATION

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The SRF/MKL1 regulatory axis is indispensable for the maturation of platelet-producing megakaryocytes (Mks). In primary hematopoietic and human erythroleukemia (HEL) cells, MKL1 over-expression enhances Mk maturation, but the mechanism by which this occurs is unknown. SRF is a ubiquitous transcription factor that can be induced by MKL1 or ternary complex factors (TCFs). We have used anti-SRF ChIP-seq and RNA-seq, to elucidate the role of SRF/MKL1 in gene regulation during Mk maturation. Using HEL cells as our model, we performed all sequencing in duplicates for differentiated and undifferentiated cells, both with and without induction of MKL1 over-expression, which promotes Mk maturation. For ChIP-seq and RNA-seq, the mean sequencing depth was 18 million and 48.5 million per population, respectively. SRF peaks were called using MACS2.0 and differential binding of SRF was analyzed using DiffBind, and differential RNA expression was analyzed with EdgeR. Our data show that in the presence of excess MKL1, a higher number of predominantly cytoskeletal genes are upregulated by the SRF/MKL1 axis. We found that phorbol ester (TPA)-induced Mk maturation with over-expression of MKL1 have 25% of upregulated genes under the influence of increased SRF binding, as opposed to 10.8% without excess MKL1. Of genes upregulated in response to TPA, most are regulated by SRF and ETS2, whereas those upregulated in the presence of TPA plus MKL1 over-expression are regulated exclusively by SRF. Matrix ChIP-PCR using anti-SRF and anti-MKL1 further validated that the two proteins have increased binding at loci proximal to their target genes, such as CORO1A, KALRN, NDRG1, and WWC1, in the presence of excess MKL1. Thus, both the SRF/MKL1 and SRF/TCF regulatory axes are involved in Mk maturation. However, in the presence of excess MKL1, the SRF/MKL1 gene regulatory axis becomes more pronounced, and upregulates genes important for enhancing the Mk maturation. Analogous studies are ongoing with primary human cells.

F-3010

LEUKEMIC MESENCHYMAL STROMAL CELL COX2-PG SIGNALING REGULATES DONOR ANTI-LEUKEMIA IMMUNITY

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Hematopoietic stem cell transplantation (HSCT) is considered the gold standard for treatment of hematologic malignancies, including Fanconi anemia (FA), a cancer-prone disease with extremely high incidence of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). However, eradication of residual leukemia stem cells (LSCs), which often contributes to relapse, remains the challenge for HSCT. Here we investigate the interaction between leukemic mesenchymal niche and donor hematopoietic stem progenitor cells (HSPCs) by modeling FA HSCT. We found that healthy donor CD34+ HSPCs cocultured on mesenchymal stromal cells (MSCs) derived from patients with AML exhibit high human engraftment characteristic of HSPC and myeloid expansion in NOD/SCID/IL-2gamma-/-/SGM3 (NSGS) mice. LC/MS-based untargeted metabolomics analysis revealed that prostaglandins (PGs), an inflammatory component of the mesenchymal secretome, are the only metabolites that are progressively elevated in MDS and AML MSCs compared to the MSCs from healthy donors or patients with cytopenias but without cancer. Inhibition of the inflammatory cyclooxygenase 2 (COX2) in the AML MSCs ex vivo ameliorates HSPC and myeloid expansion in transplanted recipients of the cocultured CD34+ cells. In addition, transcriptome analysis demonstrated dysregulation of genes involved in the NR4A family of nuclear hormone transcription factors (TFs) and the WNT/beta-catenin signaling pathway in the CD34+ cells co-cultured on MSCs derived from AML patients. Consistently, reduced MSC secretion of PGs subsequent to inhibition of COX2 leads to a significant decrease in the expression of the NR4A TFs and the WNT signaling genes including Wnt ligand WNT5A, beta-catenin (CTNNB1) and the WNT effector LEF1 in cocultured CD34+ cells. Furthermore, knocking down the NR4A TFs or CTNNB1 abrogated the expansion of progeny of the AML MSC-cocultured HSPCs in recipient mice. Together, these findings suggest that specific interactions between leukemic mesenchymal niche and donor HSPCs orchestrate a novel COX2/PG/NR4A signaling axis, connecting inflammation, cellular metabolism and cancer immunity.

F-3012

THREE DISTINCT POOLS OF STEM CELLS SWITCH BEHAVIOR TO MAINTAIN AND REPAIR THE MOUSE INCISOR EPHITELIUM

Sharir, Amnon[1], Mian, Wan[1], Siebel, Christian[2] and Klein, Ophir[1]

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Maintenance and repair of continually shedding epithelial tissues is coordinated by stem cells that possess the capacity for both prolonged self-renewal and generation of a continual supply of differentiated cells. The mechanism by which cell requirements are met during homeostasis and repair remains a critical open question in the field of stem cell biology. Classical models long posited a proliferative hierarchy with eternal, long-lived stem cells giving rise to transit-amplifying cell(s) with limited proliferative potential, but an abundance of recent work indicates that in many organs equipotent progenitor cell populations maintain and repair tissues without support from a reserve stem cell pool. In this study, we investigated cellular dynamics in the ever-growing mouse incisor, an emerging model that benefits from an exceptionally large pool of stem cells. We have found that three distinct pools of epithelial cells with different proliferative dynamics maintain the incisor enamel during homeostasis and response to tissue damage. In contrast to previous assumptions that normal renewal relies on long-lived stem cells at the apex of a differentiation hierarchy, our lineage analysis and mathematical modeling revealed that a relatively large pool of proliferating progenitors resides in the proximal region of the tooth and drives homeostatic turnover. These progenitors divide regularly to generate differentiated ameloblasts, whereas the quiescent cells in the outer epithelium – previously thought to be the main drivers of renewal – do not contribute to homeostasis. Using BrdU and H2B-GFP label-dilution assays, we discovered that slowly dividing cells in a monolayer of cells called the stratum intermedium, which underlies the differentiating ameloblasts, persist during the lifetime of the animal and contribute to both maintenance and repair. Catastrophic injury activates cells at the outer enamel epithelium that do not participate in tooth maintenance to repopulate the niche. Together, these findings upend the current theory regarding the location and dynamics of incisor ephithelial stem cells and establish several important features of stem cell heterogeneity that may apply to other stem cell-supported adult tissues.

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F-3014

TWO SIDES OF THE SAME COIN: MESENCHYMAL STROMAL CELLS IN EXPERIMENTAL LUNG INJURY

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The acute respiratory distress syndrome (ARDS) remains a major challenge in the management of critically ill patients. The bone marrow derived-mesenchymal stromal cells (MSCs) have been shown to be protective given shortly after the endotoxin-induced ARDS is established in animal models, likely through the paracrine anti-inflammatory effects of MSCs. We therefore hypothesized that early administration of MSCs would be beneficial too in other models of ARDS. We used a model of the intratracheal instillation of HCl or/and mechanical ventilation induced-lung injury in mice followed 48h later by intratracheal and intravenous administration of MSCs. We observed that the treatment with MSCs further deteriorated the HCl-induced injury while attenuated the ventilator-induced lung injury. Approaches of either preconditioning the lung by increasing antioxidant capacity prior to MSC therapy, or administration of MSCs packaged with human gene encoding IL-10 or human hepatocyte growth factor, decreased the HCl-induced lung injury. These results suggest that MSC therapy can result in both protective and deleterious effects, which is determined by local microenvironment at the time of administration. Identification and modification of the microenvironment may be crucial for MSC therapy in ARDS.

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F-3016

MODELING THE FETAL BONE MARROW NICHE IN ZEBRAFISH AND TRACKING ESTABLISHMENT OF THE ADULT HEMATOPOIETIC STEM CELL POOL

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Hematopoietic stem and progenitor cells (HSPC) support the entire blood system throughout life. This small number of cells are produced exclusively during development. In all studied vertebrates, HSPC emerge from the hemogenic endothelium of the dorsal aorta, migrate to and expand in the fetal liver, then migrate again to colonize the presumptive adult niche--this is the fetal bone marrow in mammals and the kidney marrow in zebrafish. In mammals, these events occur in utero deep in the long bones of the fetus and therefore cannot be directly observed. A better understanding of these events will reveal how a long-term stem cell pool is established. Using transparent HSPC-specific transgenic zebrafish lines (Runx:GFP and Runx:mCherry), we applied lightsheet microscopy to rapidly acquire deep tissue image stacks of the entire niche over time. In combination with endothelial-specific transgenic reporters, we observed interactions between HSPC and the perivascular niche. We previously described in the zebrafish equivalent of the fetal liver, the caudal hematopoietic tissue, that endothelial cells remodel around a single Runx:GFP+ HSPC to form a surrounding pocket. We have now found that a similar cellular structure forms in the presumptive adult niche. We reported before that dynamic quantification of live HSPC in the kidney marrow, as marked by the more broadly progenitor-expressed Runx:mCherry line, showed a relatively constant number of ~100 total HSPC. This appeared as a dynamic equilibrium of HSPC ingress and egress with occasional cell divisions. To increase optical resolution and resolve every single Runx:mCherry+ HSPC in the kidney, we used a tissue-clearing method on fixed embryos—we can now update our total estimated number to ~250 HSPC at 5 days post fertilization. Consistent with recent reports of native hematopoiesis in the mouse, a relatively small number of HSPC are established during development, and remain constant throughout life. Our zebrafish model reveals the earliest colonization of a future adult stem cell niche, and will allow functional studies of how the stem cell pool is used during homeostasis and stress.

Funding Source: NIH NIDDK K01 Research Scientist Development Award and American Society of Hematology Junior Faculty Scholar Award

F-3018

THE N-GLYCAN BRANCHING PATHWAY ALTERS NEURAL STEM CELL BIOPHYSICAL PROPERTIES AND SHIFTS FATE POTENTIAL TOWARDS ASTROGENESIS

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Neural stem cells have the potential to treat many neurological diseases and injuries due to their ability to secrete beneficial factors and to form the major cell types that comprise the central nervous system. However, these cells generate a heterogeneous population of neural stem and progenitor cells (NSPCs) when expanded in vitro, which can confound transplant outcomes. A better understanding of the cellular characteristics defining or regulating distinct progenitors in the lineage is important for predicting or controlling the types of cells formed from a population of NSPCs. We found previously the electrophysiological property membrane capacitance distinguishes neurogenic and astrogenic progenitors and hypothesized that cell surface glycosylation may affect membrane capacitance. Glycosylation modifies the structure, retention, and function of almost all proteins on the cell surface, thus influencing their interactions with extracellular cues. A gene array identifying glycosylation enzyme expression levels in E12 (neurogenic) and E16 (astrogenic) mouse NSPCs revealed differences between the two in N-glycan branching enzymes. Further analysis showed higher activity in the branching pathway in E16 NSPCs compared to E12 NSPCs in vitro and in vivo. Enriched astrogenic progenitors expressed higher levels of N-glycan branching enzymes than did unsorted controls. Supplementing E12 NSPCs with N-acetylglucosamine (GlcNAc) to drive the branching pathway increased both highly-branched N-glycans on the cell surface and membrane capacitance. GlcNAc treatment amplified

astrocyte generation at the expense of both neuron and oligodendrocyte formation, showing that altering highly-branched N-glycans affects fate choice. GlcNAc can be utilized in multiple metabolic processes, so N-glycan branching was blocked with kifunensine to test whether GlcNAc's effects on fate are dependent on this pathway. Kifunensine and GlcNAc co-treated cells did not form highly-branched N-glycans and did not exhibit the same effects on fate as GlcNAc treated cells. These data show that the effect of GlcNAc on cell fate is mediated by the formation of highly-branched N-glycans and identify the N-glycan branching pathway as a significant regulator of membrane capacitance and fate choice in the neural lineage.

F-3020

ENHANCING MATURATION OF IN VITRO HUMAN CORTICAL NEURONS

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In vitro derived cortical human neurons produced with current protocols lack dendritic spines, which constitute the primary post-synaptic aspect of synapses where most excitatory connections in the brain occur. The absence of mature synapses and spines presents a significant roadblock to realizing the full potential of these cellular resources for advancing therapeutic discovery. Our objective is to identify factors that can enhance synaptogenesis and/or spineogenesis in in vitro derived human cortical neurons. We will establish a screening assay to achieve dendritic spine development in a dish. To generate excitatory cortical neurons in vitro, human pluripotent stem cells are infected with TetO-Ngn2-T2A-Puro and Ubiq-rtTA lentivirus and treated with doxycycline to induce ectopic Ngn2 expression, followed by puromycin to eliminate uninfected cells. We are currently establishing assay parameters for quantification of synapse and spine formation by optimizing cell plating formats, immunophenotyping and data analyses. Chemical modification of synapse or spine formation has the advantage of being practical to incorporate into neuron differentiation protocols across many different hPSC lines allowing us to conduct pilot screens for candidate factors that enhance synaptogenesis and/or spineogenesis in human neurons. Enhancement of synaptogenesis in in vitro derived cortical human neurons would enable mechanistic studies not currently feasible in human neurons. The ability to generate human neurons with bona fide dendritic spines would transform our ability to dissect synaptic mechanisms foundational for information processing in the brain and disrupted in a host of brain diseases.

F-3022

TOPOISOMERASE 3B-KNOCKOUT MICE DISPLAY PHENOTYPES OF SCHIZOPHRENIA INCLUDING DEFECTIVE NEUROGENESIS AND INCREASED ANXIETY

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Topoisomerase 3b (Top3b) represents a new class of topoisomerases that can catalyze changes of topology for both DNA and RNA. Increasing evidence suggests that Top3b interacts with the Fragile X mental retardation syndrome protein, FMRP, to regulate mRNA metabolism and promote synapse formation. Individuals carrying deletion or de novo single nucleotide variants of Top3b gene are at increased risk of developing schizophrenia, autism and intellectual disability. However, the functions and pathologic mechanism of Top3b in mental disorders are unclear. Here we show that Top3b-deficient mice display several phenotypes that have been observed in schizophrenia patients and animal models. These include: increased anxiety and intensified fear conditioned memory in several behavioral tests; enlarged ventricles; reduced proliferation and differentiation of adult neural stem cells in subventricular zones (SVZ) and hippocampus; and increased levels of mental disorder-associated hormones such as growth hormone and arginine vasopressin. Mechanistically, Top3b binds to a group of mRNAs, which are crucial for adult neurogenesis and newly developed tissue structure; and may regulate emotional conditions including anxiety level and fear memory. Our data support the notion that Top3b mutation is a risk factor for schizophrenia. We propose that Top3b inactivation results in reduced expression of hormones important for neurodevelopment, leading to defective adult neurogenesis, abnormal anxiety, and mental dysfunction.

F-3026

INVESTIGATING THE ROLE OF SYNGAP1 IN HUMAN STEM-CELL DERIVED EXCITATORY NEURONS

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The current models for investigating psychiatric or neurodegenerative diseases rely on animal and/or neuronal cell culture based platforms. While these formats have been integral for elucidating many underlying disease mechanisms, this often leads to a failure to translate into effective therapeutics. Many of these failures can be attributed to the biological differences between human disease and animal/cell models. Our lab has pioneered a rapid and robust procedure for differentiating human stem cells into functional excitatory cortical neurons, providing a species-specific model. Human disease relevancy can be achieved by reprogramming patient cells into iPSC or CRISPR edited lines. This allows basic biological investigation into complex psychiatric diseases, where information is lacking. One such psychiatric disease is that caused by mutations in Synagap1, which causes a spectrum of intellectual disability, autism, epilepsy and has been linked to schizophrenia. Syngap1 is a post-synaptic density protein, where it influences the plasticity of the post-synaptic structure and embeds AMPA receptors into the post-synaptic membrane, which is mediated by NMDA signaling. Syngap1 interacts with many different proteins in the post-synaptic density; such as SHANK3, PSD-93, PSD-95 and AMPA/ NMDA subunits, which have all been implicated in psychiatric disease. It has been shown that mutations in the aforementioned Syngap1 interacting proteins affect individuals in a similar way, leading to the hypothesis that many of these post-synaptic density proteins may converge on a common disease pathway. However, it is unknown how mutations in Syngap1 affect human neuronal differentiation, morphology (dendritic and synaptic structure), excitability or downstream effects on interacting proteins. We investigated the morphological and electrophysiological changes in Syngap1 heterozygous KO human neuron lines (achieved by CRISPR editing) to better understand how these mutations contribute to disease. Syngap1 interacting proteins (SHANK3, etc.) and their expression was also examined, due to the hypothesis of converging disease pathways.

F-3028

SHIFTING CELL FATE IN THE MIDBRAIN: IMPLICATIONS FOR DEVELOPMENT AND DISEASE

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Lesch-Nyhan disease (LND) is a genetic disease characterized by neurological and behavioural disturbances, including dystonia and a compulsion to self-harm. LND is caused by mutations in the housekeeping gene HPRT1, which encodes the protein HPRT, an enzyme involved in the production of purines. Yet, despite the ubiquitous expression of HPRT, and the neurological symptoms that result from its absence, most neurons tolerate a loss of HPRT. Multiple lines of evidence have suggested that LND is instead caused by a loss of dopaminergic neuronal function without a loss of cell density. To create a novel model of HPRT deficiency, we generated induced pluripotent stem cells (iPSCs) from patients with LND that had deficient HPRT activity and control patients. We differentiated these iPSCs into dopaminergic progenitor cells (DPCs), and assessed their gene expression profiles using RNAseq. To our surprise, DPCs from HPRT-deficient patients had a gene expression pattern that diverged from normal dopaminergic development and showed increased expression of genes related to the development of glutamatergic neurons. We then generated mature, electrically active neurons from these progenitor cells. Mature neurons from HPRT-deficient patients had reduced expression of dopaminergic markers, and a concurrent increase in glutamatergic markers, and produced significantly less dopamine and substantially more glutamate than neurons derived from control patients. We have shown that this phenotype can be replicated by growing control DPCs in the presence of a HPRT blocker, or by knocking out HPRT1 using CRISPR-CAS9 gene editing. Research is ongoing to confirm similar effects in post-mortem brains of LND patients, and to elucidate the specific downstream effects of HPRT1 mutations that lead to this shift in cell fate. These results suggest that the deficit in dopaminergic neurons observed in LND patients may be caused by HPRT1 mutations shifting the cellular fate of midbrain dopaminergic cells. On a more fundamental level, these results also suggest that the cellular fate of midbrain dopaminergic neurons can be influenced by changes in purine metabolism, and that that neural cell fate is more intimately coupled to basic metabolic processes than is currently appreciated.

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F-3030

DRIVERS OF HIGH GRADE SEROUS OVARIAN CARCINOMA

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Ovarian cancer is a complex disease consisting of several genetically distinct subtypes. High grade serous ovarian carcinoma (HGSOC) is the most lethal subtype, largely due to late diagnoses, imprecise screening methods, and frequent metastasis. While no single mutation explains HGSOC initiation, common pathway mutations and alterations have recently been identified through the Cancer Genome Atlas Research Network. These include retinoblastoma (RB) (67% of cases), PI3K/Ras (45%), and Notch (22%) signaling pathways, along with genes involved in homologous recombination DNA repair (51%). Though these genes and pathways are associated with cancer incidence, correlations are unable to differentiate initiating (driver) mutations from downstream (passenger) mutations. The HGSOC cell of origin also remains uncertain, with candidate populations including the ovarian surface epithelium, fallopian tubal epithelium, and stem cell niches. To address these issues, we will perform random, combinatorial mutagenesis of 20 HGSOC-associated genes on putative cells of origin using a "minilibrary" of lentiviral CRISPR/Cas9 (LentiCRISPRv2) constructs. Cells will be isolated, randomly edited, and injected into the ovarian fat pad of mice, allowing for in vivo transformation of cells with initiating mutations. Tumors will be characterized via identification of initiating LentiCRISPRs by qPCR, next generation sequencing, histology, and protein analyses. Cell migration assays will also be performed to identify mutations associated with increased cell mobility. We hope that our results will provide significant insight into the origin of HGSOC and contribute to early detection and diagnosis.

Funding Source: NYSTEM

F-3032

THE ROLE OF RNA POLYMERASE II PAUSING IN REGULATING MOUSE EMBRYONIC STEM CELL DIFFERENTIATION

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Promoter-proximal pausing of RNA Polymerase II (Pol II) is a tightly controlled step during transcription of most genes. Pol II pause requires the pause factors DSIF and NELF, which are phosphorylated along with Pol II for productive elongation. The function of paused Pol II during embryonic development remains to be fully characterized. Our work makes use of mouse embryonic stem cells (mESCs) as a model for embryonic differentiation. Previous studies have revealed a conserved crucial residue in the DSIF subunit Spt5 that is required specifically for the pausing function of DSIF in vitro. Here we introduce a mutation of this residue (V1008D) into the endogenous spt5 locus in mESCs using CRISPR/Cas9 in an attempt to make pausing mutant mESCs. The homozygote mutant clones retained ESC morphology with normal expression of pluripotency markers, suggesting that Pol II pausing is not required for ESC identity. In contrast, mutant stem cells were less efficient to differentiate. Embryoid body (EB) differentiation studies revealed that mutant-derived EBs exhibited growth retardation and defective terminal lineage differentiation. Single-cell RNA sequencing of day10 EBs showed a persistent expression of early differentiation markers and complete loss of Hox gene activation. Global run-on sequencing (Gro-seq) of WT and mutant ESCs revealed a genome-wide reduction of paused Pol II in the mutant ESCs, confirming the attenuated pausing by the mutation. The reduction of pausing leads to increased Pol II in the gene body of some genes (referred as up-regulated genes) but reduced Pol II along other genes (referred as down-regulated genes). By comparing with ChIP-seq and ATAC-seq data, we found that down-regulated genes tend to have less permissive chromatin state with enriched repressive marks comparing to up-regulated genes, suggesting that these genes may rely more on paused Pol II to maintain chromatin accessibility for productive elongation. Thus our study identified an important role of Pol II pausing in regulating ESC differentiation and revealed a tight correlation between pausing and chromatin state, suggesting that Pol II pausing can both positively and negatively influence transcription depending on the local chromatin environment.

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F-3034

IDENTIFICATION OF CELLULAR DIVERSITY AND NEURONAL NETWORK DYNAMICS IN PHOTOSENSITIVE HUMAN BRAIN ORGANOIDS

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In vitro models of the developing human brain such as 3D brain organoids offer an unprecedented opportunity

to study aspects of human brain development and neurodevelopmental disorders in a format amenable to large-scale production and genetic engineering. However, it remains undefined what cell populations are generated within organoids and to what extent brain organoids recapitulate the regional complexity, cellular diversity, and circuit functionality of the human brain. Here, we analyzed gene expression in over 80,000 individual cells isolated from 31 human brain organoids developed for 3-6 months. We find that organoids can generate a broad diversity of cells, which we show are related to known endogenous classes, including subpopulations of neurons and progenitors of the cerebral cortex. Organoids could be developed over extended periods (over 9 months) enabling unprecedented levels of maturity including the formation of dendritic spines and of spontaneously-active neuronal networks. We found that organoids also generated many cell types of the retina and that the activity of neurons within organoids could be controlled using light stimulation of photoreceptor-like cells. These results indicate that 3D human brain organoids are capable of generating extensive cellular diversity and may offer ways to probe in vitro the functionality of human neuronal circuits using physiological sensory stimuli.

F-3036

RECAPITULATION HEPATITIS B VIRUS-HOST INTERACTIONS IN LIVER ORGANOIDS GENERATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Although vaccines and antivirus therapies against hepatitis B virus (HBV) have been improved in recent decades, about 400 million people are infected with hepatitis B worldwide, and a million people die per year from complications of hepatitis B. Many various models have been proposed for studying hepatitis B virus (HBV) infection in vitro; however, the application of these models was hampered by their cell sources, cell characteristics, and inability to reproduce the liver cytoarchitecture. Human induced pluripotent stem cells (hiPSCs), reprogrammed from somatic cells, have created unprecedented opportunities for effective differentiation into hepatocytes. To improve the hepatic cells differentiation and deeply understand the virus–host interaction, we aimed to establish 3D liver organoids (LOs) with mesenchymal and endothelial cells to reproduce HBV infection and investigate virus–host interactions during infection. By simulating liver organogenesis microenvironment, hiPSCs endoderm, mesenchymal and endothelial cells, were combined in a micro-pattern cultural system, self-organized LOs gradually developed and differentiated into more mature hepatic cells than the conventional hiPSC-derived hepatic-like cells and displayed a human liver cytoarchitecture and functions. Moreover, hiPSC-LOs were susceptible to HBV infection, with surface expression of the Na+-taurocholate cotransporting polypeptide, and could maintain long-term HBV replication. To comprehensively understand the virus–host interaction, we infected hiPSC-LOs with titers of HBV imitating acute hepatitis. The results indicated that pyroptosis was induced and hepatic functions were downregulated in hiPSC-LOs treated with a high titer of virus. We also found that the virus could enhance the epithelial-to-mesenchymal transition and affect the ultrastructure of hiPSC-LOs. To the best of our knowledge, this is the first study reproducing HBV life cycle and liver physiology changes during infection in organoids, suggesting that hiPSC-LOs might be a useful system for HBV biology research. Furthermore, many potential applications are expected for this model because of advantages of iPSCs and a chemically defined differentiation process, which makes it easy to manipulate and modify this model compared with other models.

F-3040

ENHANCED ELECTRONIC TABLET-BASED INFORMED CONSENT ENABLES TWO-WAY CONSENT PROCESS (PROVIDER-PATIENT), REAL-TIME FEEDBACK AND PROCESS EVOLUTION; MAY INCREASE PATIENT SATISFACTION

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Clinical trial patients of the UC San Diego CIRM Alpha Stem Cell Clinic desire modern approaches to care, such as during study consent and education. Understanding the educational and logistical concerns of clinical trial patients is hampered by simple discussions and paper forms. Thus, this clinical trial communication should evolve into a 2-way, modern process from the current model of provider-to-patient to additionally include the reverse (patient-to-provider). While provider-to-patient exists, quantifiable feedback from patients is rare regarding both the consenting process and patient educational materials. With these communication needs in mind, we have worked with disease-specific experts to provide enhanced electronic educational materials and consent in partnership with stem cell study experts (Alpha Clinic). To quantify these data, we have developed integrated IRB-approved research projects. We present our process and interim findings along with the noted achievements of our dynamic approach.

While our studies already offer IRB-approved e-consent on iPad tablets, we recently obtained IRB permission to record patients' annotations on these enhanced consent documents, including highlighted phrases and specifically written notes. These metadata provide both instantaneous and long-term feedback to the provider and to the Alpha Clinic reviewers. In real-time, providers are alerted to spend extra review of areas annotated by their patients, possibly increasing feelings of patient-empowerment. Over time, trends will point to areas for improvement in both educational documents and ICFs within and across stem-cell therapies. These evidence-based concerns, once translated into new materials, may pre-emptively address patients' study and disease (educational) concerns. Of note, this "annotation study" is independent of any individual stem cell clinical trial. In other words, it may be referenced by used in existing or new IRB projects, and a waiver of documented consent allows for simple integration, streamlining this newly imagined dual-direction consent process (provider-to-patient and vice-versa). Our annotation study has been incorporated into the highest-recruiting study our clinic (cirmtuzumab) and has been welcomed by sponsors for two additional, forthcoming studies.

F-3042

ANALYSIS OF THE TRENDS OF INDUCESD PLURIPOTENT STEM CELL TECHNOLOGY INNOVATION BASED ON PATENT

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In this paper, we metrologically analyze the trend of the patent applications for iPSCs over the past decade (2006-2016), and predict the future directions and clinical impacts of iPSCs based on the historical and cutting-edge developments of the leading research institutions. The overall trend analysis demonstrates that the iPSCs technology has been developing rapidly. Besides, we jointly use CiteSpace, ThemeScape Map and cluster analysis to identify and visualize the hotspots and emerging trends in iPSCs patent applications. Results show that the reprogramming/transcription factors, the molecular mechanism of reprogramming, the reprogramming efficiency and the iPSCs differentiation are the hotspots in the basic research of iPSCs, while the applied research mainly focuses on the construction of animal/disease model, drug R&D, and cell therapy. Last, we recognize the Kyoto University as the leading institute in patent citations, the size of patent families and patent litigation. According to the development history, the research focus in iPSCs of the Kyoto University has been shifted from the optimization of induction and culture technologies to the developments of differentiation methodology that induces iPSCs research into pancreatic cells, neurons, and hepatic lineage cells, and to the exploration of the culture of pancreatic islets. Moreover, the Kyoto University has cooperated with STEMCELL Technologies Inc. and Minerva Biotechnologies Inc. to promote the commercialization of iPSCs technology. The study reveals the current research status of iPSCs, predicts its future development trends and provides references for decision making on new technology R&D and future industry development in related fields.

F-3044

SUPEROVULATORY RESPONSES IN CYNOMOLGUS MONKEYS (MACACA FASCICULARIS) DEPEND ON THE INTERACTION BETWEEN DONOR STATUS AND SUPEROVULATION METHOD USED

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Superovulation methods have been extensively studied in a variety of experimental animals to aid in the development of assisted reproductive technologies (ARTs); however, the factors that influence superovulation efficiency in non-human primates (NHPs) have been the focus of little research. Thus, the current study was performed to investigate the effect of oocyte donor status, including age and body weight, on metaphase II (MII) oocyte recovery using two superovulation methods in cynomolgus monkeys. The use of Method A [recombinant gonadotrophin (75 IU/kg, 3×, 3-day intervals) and human chorionic gonadotropin (hCG)] led to great increases in ovary size and the mean number of MII oocytes retrieved in age- and body-weight-dependent manner; in contrast, both the parameters were similar in Method B [recombinant gonadotrophin (60 IU, twice daily, 6 days), recombinant gonadotropin and recombinant human luteinizing hormone (rhLH) (60 IU, twice daily, 3 days), and hCG]. Importantly, Method A showed maximal MII oocyte recovery rate in > 60-month-old or 4.5–5.0-kg female monkeys, whereas Method B was equally effective regardless of the donor age and body weight. These results indicate that superovulatory responses depend on the interaction between oocyte donor status and the superovulation method used in cynomolgus monkeys.

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F-3046

ROLE OF TELOMERIC PROTEINS IN PLANARIAN HOMEOSTASIS AND REGENERATION

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The planarian flatworm is an emerging model that is useful for studying homeostasis and regeneration due to its unique adult stem cells (ASCs). Previously, planaria were found to share mammalian TTAGGG chromosome ends and telomerases; however, their telomere protection proteins have not yet been identified. In Schmidtea mediterranea, we identified a homologue of the human protection of telomeres 1 (POT1) with an OB-fold (SmedOB1). SmedOB1 is evolutionarily conserved among species and is ubiquitously expressed throughout the whole body. Feeding with SmedOB1 double-stranded RNAs (dsRNAs) led to homeostasis abnormalities in the head and pharynx. Furthermore, several ASC progeny markers were downregulated, and regeneration was impaired. Here we found that SmedOB1 is required for telomeric DNA-protein complex formation and it associates with the telomere TTAGGG sequence in vitro. Moreover, DNA damage and apoptosis signals in planarian were significantly affected by SmedOB1 RNAi. We also confirmed these phenotypes in Dugesia japonica, another flatworm species. Our work identified a novel telomere-associated protein SmedOB1 in planarian, which is required for planarian homeostasis and regeneration. The phylogenetic and functional conservations of SmedOB1 provide one mechanism by which planarians maintain telomere and genome stability to ensure their immortality and shed light on the regeneration medicine of humans. We also identified some other telomeric proteins in planarian and explored their roles in homeostasis and regeneration.

F-3048

TEC CONTROLS PLURIPOTENCY AND EARLY CELL FATE DECISIONS OF HUMAN PLURIPOTENT STEM CELLS VIA REGULATION OF FGF2 SECRETION

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Human pluripotent stem cells (hPSC) require signaling provided by fibroblast growth factor (FGF) receptors. This can be initiated by the recombinant FGF2 ligand supplied exogenously, but hPSC further support their niche by secretion of endogenous FGF2. In this study, we describe a role of TEC kinase (tyrosine kinase expressed in hepatocellular carcinoma) in this process. We show that TEC-mediated FGF2 secretion is essential for hPSC self-renewal and its lack mediates specific differentiation. Following both shRNA- and siRNA-mediated TEC knock-down, hPSC secrete less FGF2. This impairs hPSC proliferation which can be rescued by increasing amounts of recombinant FGF2. TEC downregulation further leads to lower expression of the pluripotency markers. While differentiation into embryoid bodies and hepatic endoderm remains unaffected, hPSC with downregulated TEC more efficiently differentiate into neuroectodermal lineage. In contrast, the hPSC with downregulated TEC fail to efficiently form cardiac mesoderm. Our data thus demonstrate that TEC is yet another regulator of FGF2-mediated hPSC pluripotency.

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F-3050

THE EFFICIENCIES OF IMSC GENERATION DIFFER AMONG THE PARENTAL IPSC SOURCE

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Mesenchymal stem cells (MSCs) have tremendous potential for cell-based therapeutic applications. These cells can be collected from multiple tissues (bone marrow, umbilical cord blood and adipose tissue), and requires invasive procedures in harvesting, which causes considerable donor variations and limited expandability.

To circumvent these issues, induced pluripotent stem cell (iPSC) -derived MSC (iMSC) may provide an alternative, but it is not clear to what extent they are similar or better compared with those from MSCs. Here we performed a comparative analysis for characterizing iMSCs, using conventional MSC assessment markers. iMSCs were generated from iPSCs derived from fibroblast (Fib), peripheral blood mononuclear cell (PBMC) and umbilical cord tissue derived MSC (UCMSC), respectively. Fib-iPSC-iMSC, PBMC-iPSC-iMSC and UCMSC-iPSC-iMSC showed typical MSC morphology and MSC-typical surface marker profile, and they were able to differentiate into osteogenic, chondrogenic, and adipogenic lineages. Gene expression analysis revealed that iMSCs were enriched with mesenchymal genes. Fib-iPSC-iMSC, PBMC-iPSC-iMSC and UCMSC-iPSC-iMSC displayed typical MSC morphology, and positive for MSC cell surface markers such as CD73, CD90 and CD105. We also found that both the proliferation rate and differentiation potential of iMSC derived from UCMSC-iPSC were significantly higher ($p < 0.01$) than those from PBMC-iPSC and Fib-iPSC. Finally, compared with the Fib-iPSC-iMSC and PBMC-iPSC-iMSC, UCMSC-iPSC-iMSC displayed a unique expression pattern of mesenchymal lineage genes and was well responsive to iPSC-MSC differentiation protocols. Our results demonstrate that the iPSC source impacts the cellular properties of iMSC. UCMSC-iPSC might be the best option for iMSC establishment regarding their differentiation potential.

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F-3052

LOSS OF CCBE1 AFFECTS CARDIAC-SPECIFICATION AND PROLIFERATION IN DIFFERENTIATING MOUSE EMBRYONIC STEM CELLS

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Understanding the molecular pathways regulating cardiogenesis is crucial for the early diagnosis of heart diseases and improvement of cardiovascular disease. During normal mammalian cardiac development, collagen and calcium-binding EGF domain-1 (Ccbe1) is expressed in the first and second heart field progenitors as well as in the proepicardium, but its role in early cardiac commitment remained unknown. We here demonstrate that during mouse embryonic stem cell (ESC) differentiation Ccbe1 is upregulated upon emergence of Isl1- and Nkx2.5- positive cardiac progenitors. Ccbe1 is markedly enriched in Isl1-positive cardiac progenitors isolated from ESCs differentiating in vitro or embryonic hearts developing in vivo. Disruption of Ccbe1 activity by shRNA knockdown or blockade with a neutralizing antibody results in impaired differentiation of embryonic stem cells along the cardiac mesoderm lineage. In addition, knockdown of Ccbe1 leads to reduced cell proliferation resulting in smaller embryoid bodies. Furthermore, full-lenght recombinant CCBE1 protein was shown to partially rescue the phenotype in differentiating Ccbe1 KD ESCs. Collectively, our results show that CCBE1 is essential for the proliferation and formation of cardiac mesoderm in differentiating mouse ESCs.

Funding Source: This work was supported by FCT in the frame of projects PTDC/SAU-ENB/121095/2010 and HMSP-ICT/0039/2013; from IBB/CBME, LA to J.A.B. in the frame of Project No. PEst-OE/EQB/LA0023/2013. From iNOVA4Health - UID/Multi/04462/2013.

F-3054

GENERATION OF T REGULATORY CELLS FROM MULTIPLE SCLEROSIS PATIENT-SPECIFIC IPS CELLS

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Multiple Sclerosis (MS) is an autoimmune-mediated neurodegenerative disease. Current medications are available that benefit for the relapsing phases of the disease but may be associated with potentially serious side effects. T regulatory cells have been investigated in clinical trials for their safety and therapeutic potential in solid organ transplant or hematopoietic stem cell recipients and patients that suffer from autoimmune diseases. Here, we propose the derivation of T regulatory cells from MS patient-specific iPSC lines to establish a novel and potentially beneficial approach to target the autoimmune inflammation in MS patients by

immunotherapy. T regulatory cells differentiated in vitro from human iPS cells offer a number of key advantages for therapy over using ex vivo expanded T regulatory cells. Patient-specific iPSCs can be precisely genetically engineered and they represent an inexhaustible supply of autologous cells for therapy. Our aim is to identify the best possible candidates of T regulatory cells for future therapy and define parameters for their efficient derivation in vitro and we have made significant advances toward this goal. We generated patient-specific iPS cell lines from multiple sclerosis patients representing the two main subtypes of MS, relapsing-remitting and primary progressive MS. We differentiated these lines to CD34+ hematopoietic stem cells and succeeded in deriving CD3+ T lymphocytes. This is a crucial step towards our goal to generate single positive CD4+ T cells that we can convert into T regulatory cells. However, generation of a homogeneous culture of differentiated cells is a key objective and remains challenging. To this end, we have succeeded in manipulating MS patient-specific iPSCs with the CRISPR/Cas system. Specifically, we are in the process of introducing reporter alleles into genes encoding for key regulators of lymphoid development that will enable us to enrich differentiation cultures by using flow cytometry. In addition, it is known that FOXP3 is the master transcription factor of T regulatory cells. Therefore, we have generated a MS patient-specific iPSC line that carries an eGFP reporter in the endogenous FoxP3 locus to enrich for cells that turn on a T regulatory transcriptional profile during differentiation.

F-3056

PLANT DERIVED NANOFIBRILLAR CELLULOSE (NFC) HYDROGEL SUPPORTS ROBUST HUMAN NEURONAL NETWORK FORMATION IN VITRO

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Human neuronal cultures are promising tools for studying e.g. disease mechanisms, drug response or developmental biology. However, traditional flat 2D cultures on top of rigid surface fail to offer in vivo like niches to the cells. 3D cell cultures are able to overcome some of the limitations occurring in 2D cultures by offering cells artificial extracellular matrix (ECM) and more in vivo mimicking environment. Highly biocompatible nanocellulose based hydrogels have proven to be potential for 3D culturing of various cell types. These plant derived nanofibrillar cellulose (NFC) hydrogels mimic native soft tissue ECM in fiber size and in mechanical properties, thus providing cells more in vivo like growth environment. In this study pre-differentiated human pluripotent stem cell derived neurons where cultured as encapsulated within the GrowDex® NFC hydrogels from UPM Kymmene Oyj (Finland). Experiments were performed with concentration of five million cells per ml of hydrogel and cells were cultured for two weeks. The formation of neuronal networks inside the hydrogels was evaluated by immunocytochemical staining against neuronal markers after the two weeks with confocal imaging. Sample preparation with these one component hydrogels was convenient and the prepared 3D cultures had homogenous cell distribution. During two weeks culturing period, a robust neurite outgrowth was observed in all dimensions. In best cases, the neuronal network filled remarkable volume of the whole hydrogel block. Based on these results it can be concluded that studied hydrogels offered very good growth environment for human neural cells and the NFC hydrogel based neural cultures should be studied further.

Funding Source: This study has been funded by UPM Kymmene Oyj (Finland) and by TEKES (Finnish Funding Agency for Technology and Innovation) Human Spare Parts project.

F-3058

MIDBRAIN TRANSCRIPTION FACTORS LMX1A, FOXA2, & OTX2 DRIVE NEURONAL DEVELOPMENT IN THE SUBSTANTIA NIGRA IN 6-OHDA RAT PARKINSON MODEL

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In general, cell replacement strategies for the treatment of Parkinson´s disease (PD) have been the transplantation of fetal dopaminergic grafts in the striatum. The striatum receives dopaminergic input from the substantia nigra (SN) which is primarily impaired in PD. Recently, our group has shown that the adult substantia nigra pars compacta harbors a robust neurogenic environment which could become the basis for novel transplantation paradigms to reduce side effects such as disabling dyskinesias. We also developed genetically modified human embryonic stem cell lines (hESC-H9) that carry key midbrain transcription factors that drive more efficient dopaminergic differentiation in vitro.In the present study, we evaluated two of these human stem cell lines, one containing the human LMX1A (L), OTX2 (O) & FOXA2 (F) gene coding sequences (hESC-H9-LOF) and control line (hESC-H9-PC) in a 6-hydroxydopamine (6-OHDA)-lesioned rat model. First, we standardized the human stem cell culture conditions to ensure consistent growth, expansion, freeze/thaw, and embryoid body formation as well as

accurate techniques to EB single cell dissociation for its transplantation of these genetically engineered lines. Second, the cells were transplanted stereotaxically in the SN of 6-OHDA rats and animals were euthanized at 7 and 15 days post transplant (dpt). Results reveled that around 50% of the H9-LOF (7 dpt) and 30% of H9-LOF (15 dpt) transplanted rats, but none of the H9-PC group developed rosettes in the graft zone, pointing that the neural differentiation process began by the initiation of a primitive neuroectoderm only in the H9-LOF. Analysis of immunofluorescence studies of specific neuronal markers of the dopaminergic differentiation pathway in 7, 15, 31 dpt are currently underway.

Funding Source: DGAPA-PAPIIT IN207116

F-3060

CRISPR/CAS9-MEDIATED EDITING OF TRINUCLEOTIDE REPEAT EXPANSION IN MYOTONIC DYSTROPHY

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CRISPR/Cas9 is an attractive platform to potentially correct dominant genetic diseases by gene editing, a concept which remains largely unproven. In the current proof-of-principle study, we explored the use of CRISPR/Cas9 for gene editing in myotonic dystrophy type 1 (DM1), an autosomal dominant disorder associated with severe myotonia and skeletal muscle dysfunction. The DM1 pathology is caused by trinucleotide CTG repeat expansion in the 3' untranslated region (UTR) of the human myotonic dystrophy protein kinase (DMPK) gene. We designed a CRISPR/Cas9-based strategy using dual guide RNAs and S. pyogenes Cas9 that specifically excises this pathogenic CTG repeat expansion in the DMPK 3' UTR. We first generated DM1 patient-specific iPSCs and subsequently induced them to differentiate into myogenic cells and myotubes. One of the hallmarks of DM1 is the emergence of ribonuclear foci that accumulate in the nucleus of patient's cells. CRISPR/Cas9-mediated excision of the triplet repeats expansion resulted in the disappearance of these ribonuclear foci that sequester MBNL1 splicing factors in the DM1-iPSC-derived myogenic cells, resulting in the normalization of the splicing pattern. This proof-of-concept study validates the use of CRISPR/Cas9 to genetically correct nucleotide repeat expansions associated with dominant genetic disorders that cause severe human pathologies.

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F-3062

SIMULATION OF OSTEOGENESIS IMPERFECTA USING INDUCED PLURIPOTENT STEM CELL AND CRISPR/CAS9 GENE EDITION

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Osteogenesis imperfecta is a genetic disease characterized by repeated bone fragility fractures. The cause of bone fragility of osteogenesis imperfect is due to defective collagen formation from mutation of COL1A1 or COL1A2. Osteogenesis imperfecta is impossible to cure with current therapeutic strategies. Induced pluripotent stem cell (iPSC) from patients with specific diseases is now utilized for disease simulation and drug screening platform. Here, we generated iPSCs from a family of osteogenesis imperfect (OI). Single gene mutation of COL1A1 gene was identified in disease family. OI-iPSCs revealed defected mineralization in vitro osteogenesis system. CRISPR/Cas9 vector was cloned for correcting mutated genes in OI-iPSCs. CRISPR/Cas9 system was successful in editing COL1A1 mutated OI-iPSCs. Gene corrected OI-iPSC showed the recovery of their osteogenic potential. Recovered osteogensis was also reproduced in vivo at calvarial bone defect model of SCID mice. OI-iPSC could help to study the core pathophysiology of this rare disease. Furthermore, CRISPR/Cas9-based gene correction may give the chance to therapeutic application on osteogenesis imperfect.

F-3066

UPREGULATION OF ENDOPLASMIC RETICULUM STRESS (ER STRESS) IN HUMAN IPSC DERIVED NEURAL CREST CELLS FROM PATIENTS WITH FUCHS ENDOTHELIAL CORNEAL DYSTROPHY

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Fuchs endothelial corneal dystrophy (FECD) is a common corneal dystrophy and a major indication of corneal transplantation. Although the detailed pathophysiology of this disease is still not understood, the involvement of endoplasmic reticulum stress (ER stress) and oxidative stress are suggested. Genetic linkages recently shown in genome-wide association study include single nucleotide polymorphisms (SNPs). We newly established human induced pluripotent stem cells (iPSCs) from six FECD patients for the investigation of this disease. Sendai virus vector was used to generate iPSCs from human blood cells. The advantage of Sendai virus vector is the high reprogramming efficiency and safety. We examined the SNPs reported in previous publications, and 3 different SNPs were found in the TCF4 gene from one patient. Next, we derived neural crest cells (NCC) from iPSCs defined as integrin alpha 4- p75 NTR double positive cells sorted by FACS. We compared C-NCC (control normal iPSCs derived NCC) and F-NCC (FECD iPSCs derived NCC). Multi array real time PCR (QIAGEN® PROFILER PCR ARRAY) was performed to pick up candidates from multiple ER stress markers. Among them, CHOP and ATF4 were shown to be highly expressed. Real time PCR showed CHOP was notably up-regulated in F-NCC compared to C-NCC. Further analysis using iPSC derived corneal endothelium cells for ER stress markers are underway.

F-3068

UNRAVELING FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY (FSHD): FROM HUMAN PRIMARY MYOBLAST TO IPSC DERIVED MYOTUBES

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Facioscapulohumeral Muscular Dystrophy (FSHD) is an autosomal dominant muscular dystrophy that results in the debilitating loss of muscle mass and strength. FSHD causes a severe loss of muscles in the face, back and extremities causing a reduced quality of life and often results in disambulation. FSHD is caused due to the deletion of a subset of D4Z4 macro-satellite repeat units present in the subtelomeric region of chromosome 4 (4q35). This deletion is known to activate expression of DUX4, encoding a transcription factor in muscle and the likely cause for FSHD pathogenesis that ultimately leads to toxicity and muscle loss. The use of human induced pluripotent stem cells (iPSCs) to generate skeletal muscle cells provides a limitless source of cells and an in vitro system to study DUX4 expression during muscle development for insights on molecular and cellular pathogenesis of FSHD. In this study, FSHD patient and healthy human primary myoblasts were reprogrammed to iPSCs. The iPSCs were differentiated into skeletal muscle cells by overexpression of MyoD which is a known master regulator of human skeletal muscle development. Myogenic marker expression was analyzed in both normal and FSHD iPS derived myotubes by immunofluorescence and quantitative PCR assay at different time points of skeletal muscle differentiation. DUX4 downstream targets like ZSCAN4 and TRIM43 were highly expressed in FSHD iPS derived myotubes but were absent in normal iPS derived myotubes. Knockdown of DUX4 expression using siRNA blocked ZSCAN4 and TRIM43 expression in the FSHD iPS-derived myotubes. The results indicate that FSHD iPSC derived myotubes can recapitulate the Dux4 dependent gene activation as seen in FSHD patient muscle. Finally, we developed a procedure for large-scale expansion of iPS derived myoblasts for high-throughput applications. This enables us to discover compounds and targets that can modulate Dux4 activation or rescue DUX4 toxicity in skeletal muscle.

F-3070

DIRECT CONVERSION OF EPITHELIAL STEM CELLS FROM HUMAN URINE CELLS BY DEFINED TRANSCRIPTION FACTORS

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Epithelial stem cells are considered as important cell source for skin or epidermal regeneration by the regenerative capacity of multipotent adult stem cell. Epithelial stem cells give promise for modeling, prevention and treatment of skin diseases and also have potential for regenerative medicine. However, the low efficient of maintaining the relevant epithelial stem cell population in cultures limited functional epithelia modeling and clinical studies. Recently, combinational overexpression of lineage-specific transcription factors can convert adult somatic cell type from one to another. Here, we focused on direct convertion of epithelial stem

cells from human urine cells, which could simply obtained by a noninvasive method, by using transcription factors. We have suggested that combination of transcription factors could enable human urine cells convert to epithelial stem cell like state, we termed as induced epithelial stem like cells (iEpSCLCs). These generated iEpSCLCs exhibited morphologically similar to primary keratinocytes and expressed epithelial stem cell and keratinocyte markers both at protein and mRNA levels. These iEpSCLCs also have high colony-forming efficiency and could be maintained at least 10 passages. Moreover, iEpSCLCs are similar to hair follicle stem cells in capacity to differentiate into mature keratinocytes and sebocytes in vitro. Overall, our results suggest that stably expandable iEpSCLCs can be directly converted from human urine cells by defined transcription factors. It will contribute to our further research for human epithelia modeling and future disease application.

Funding Source: This work was supported by the Ministry of Health &Welfare, Republic of Korea (grant No. : HI15C2944) and School of Life Sciences and Biotechnology for BK21 PLUS, Korea University.

F-3072

ENHANCING PLURIPOTENCY REPROGRAMMING BY CHEMICAL ERASURE OF SOMATIC TRANSCRIPTIONAL PROGRAM

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One critical task in pluripotency reprogramming is to erase the somatic transcriptional program of starting cells. Here, we present a new strategy that reprogramming to pluripotency can be facilitated with small molecules to interfere with the somatic transcriptional program. We show that mild chemical targeting of the acetyllysine-binding pockets of the BET bromodomains, the transcriptional bookmarking domains, robustly enhances reprogramming. Furthermore, we show that such chemical targeting of BET bromodomains downregulates or turns off the expression of somatic genes in both naïve and reprogramming fibroblasts. Chemical blocking of the BET bromodomains also results in loss of fibroblast morphology early in reprograming. In summary, pluripotency reprogramming can be facilitated by chemical erasure of somatic transcriptional activity mediated by BET proteins.

Funding Source: UAB faculty development

F-3074

GENERATION OF INDUCED PLURIPOTENT STEM-LIKE CELLS FROM WALRUS (ODOBENUS ROSMARUS)

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The walrus (Odobenus rosmarus) is a large pinniped marine mammal that resides near the Arctic Circle. Research on these animals suffers from the lack of molecular and cellular tools. Induced pluripotent stem cells (iPSCs) generated by direct molecular reprogramming of somatic cells show many biological characteristics of embryonic stem cells, including capacity for infinite proliferation, the ability to form the derivatives of all three germ layers, and the utility for germline transmission. We generated iPS-like cells from walrus skin fibroblasts and characterized their stemness. Drug-inducible expression of human OSKM (Oct4, Sox2, Klf4 and c-Myc) factors and modified cell culture conditions were critical for the development of these cells. The iPS-like cells displayed characteristic morphology, expressed pluripotent markers (alkaline phosphatase, OCT4, SOX2 and SSEA-1), formed embryoid bodies, and showed differentiation patterns typical of iPSCs. We further investigated epigenetic changes in H3K9ac and 3K27ac in walrus fibroblasts and iPS-like cells using immunofluorescence. The patterns of histone acetylation of walrus iPS-like cells were similar to those of mouse and human iPSCs. Overall, we established stable walrus iPS-like cells, which offer a molecular and cellular model for studies on this remarkable animal.

F-3076

EFFICIENT INDUCIBLE GENE MODULATION IN HUMAN PLURIPOTENT STEM CELLS AND IN THEIR DERIVATIVES

Ortmann, Daniel[1], Bertero, Alessandro[2], Matthias, Pawlowski[3], Snijders, Kirsten[1], Yiangou, Loukia[1], Cardoso de Brito, Miguel[1], Brown, Stephanie[1], Bernard, William[1], Tavares, Joana[1], Cooper, James[1], Giacomelli, Elisa[1], Gambardella, Laure[1], Hannan, Nicholas[4] Iyer, Dharini[1], Sampaziotis, Fotios[1], Serrano, Felipe[1], Zonneveld, Mariëlle[1], Pedersen, Roger[1] ,Sinha, Sanjay[1], Kotter, Mark[1] and Vallier, Ludovic[1]
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To realise the full potential of human pluripotent stem cells (hPSCs), robust ways to modulate gene expression are paramount. Here we present efficient inducible systems to knock-down, knock-out or over-express genes based on targeting of genomic safe harbours. For knock-down and knock-out applications, we combined an improved tetracycline-inducible system with either shRNA or CRISPR/Cas9 technology respectively. Importantly, the system can deliver individual or multiplexed gene knockdown or knockout in both hPSCs and multiple differentiated cell types. To showcase its functionality in various contexts, we employed this system to investigate the function of transcription factors (OCT4 and T), cell cycle regulators (cyclin Ds) and epigenetic modifiers (DPY30) during pluripotency or differentiation of hPSCs. In order to obtain an efficient over-expression platform, we optimised inducible gene expression in hPSCs using a dual safe genomic harbour targeting strategy. We then utilised this system to over-express crucial transcription factors to reprogram hPSCs directly to various mature cell types. As proof of principle, we demonstrated rapid, robust and deterministic reprogramming of neurons (iNGN2) and skeletal myocytes (iMYOD1). Finally, we developed a forward programming strategy to efficiently and expeditiously generate human oligodendrocytes. Taken together, this system enables controlled, efficient and reproducible modulation of gene expression in hPSCs and their derivatives. Employed for forward programming, new cell types can be swiftly obtained.

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F-3078

ESTABLISHMENT OF STABLY EXPANDABLE INDUCED MYOGENIC STEM CELLS BY USING FOUR DEFINE`D FACTORS

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Life-long regeneration of healthy muscle by cell transplantation is an ideal therapy for patients with degenerative muscle diseases. Yet, obtaining muscle stem cells from patients is very limited due to their exhaustion in disease conditions. Thus, development of a method to obtain healthy myogenic stem cells is required. Here, we show that the four transcription factors, Six1, Eya1, Esrrb and Pax3, are necessary to convert fibroblasts into induced myogenic stem cells (iMSCs). iMSCs showed effective myogenic differentiation and also higher proliferation capacity than MDSC. We further isolated CD106-negative and α7-integrin-positive iMSCs (sort-iMSCs) showing higher myogenic differentiation capacity than iMSCs. Moreover, genome-wide transcriptomic analysis of iMSCs and sort-iMSCs, followed by network analysis, revealed the genes and signaling pathways associated with enhanced proliferation and differentiation capacity of iMSCs and sort-iMSCs, respectively. The stably expandable iMSCs provide a new source for drug screening and muscle regenerative therapy.

F-3080

GROWTH FACTOR DEPOTS AND STABLE GRADIENTS MADE USING COMPLEX MICROCRYSTALS PROVIDE PRECISE CELL GUIDANCE

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Growth factors are important components of cell culture media and have significant therapeutic potential. Yet they suffer from inherent instability which has limited their potential in the lab and hindered their transition to the clinic. PODS™ (POlyhedrin Delivery System) growth factors address this issue by encasing bioactive growth factors in a crystalline protective shell. This structure is uniquely capable of providing long term dosing and generating stable growth factor gradients as the cargo is slowly released from the casing. PODS™ is a process which incorporates newly expressed growth factors into crystals of the Bombyx mori polyhedrin protein to form cubic polyhedrin protein crystals typically 3-10 µm across. These crystals are highly stable when stored at ambient temperature. However, once exposed to proteases, PODS™ crystals slowly dissolve and the cargo

is released providing a steady stream of active growth factor into the surrounding medium. These crystalline depots of bioactive growth factor can be used to functionalize surfaces, generate stable gradients and deliver long-term therapeutic dosing. It has previously been demonstrated that repair of bone damage in-vivo can be signi¬ficantly improved by treating animal bone with PODS™ BMP-2 compared with standard recombinant BMP-2. Here, we demonstrate the utility of PODS growth factors ™ for the formation of a stable gradient which can uniquely modify the behaviour of PC12 neuronal precursor cells. When the PODS™ crystals containing neurotrophic growth factors are spotted into a disc shape on a surface, this generates a radial gradient extending from the edge of the disc. Neuronal precursor cells migrate up the gradient, differentiate and extend neurites perpendicular to the gradient. This results in a ring structure of connected neuronal cells surrounding the PODS™ crystals. PODS™ technology has now been applied to a wide range of growth factors for which it delivers sustainable, physiologically relevant doses. PODS™ has potential to advance the expanded use of proteins in the field of regenerative medicine.

Funding Source: This work has been funded privately and by grants from JST and JSPS.

F-3082

HIGH-THROUGHPUT ASSESSMENT OF DRUG EFFECTS ON CALCIUM TRANSIENTS IN HUMAN INDUCED PLURIPOTENT STEM CELL (hiPSC) DERIVED CARDIOMYOCYTES

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Assessment of cardiac safety and efficacy of drug candidates is highly important for effective drug development. Hence, there is a pressing need for in vitro screening methods to detect cardioactive effects of compounds early in the drug discovery process. To provide a relevant in vitro model to investigate effects of drug candidates on cardiac physiology, we have recently developed hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes), which exhibit a relatively high level of maturity. We used these cardiomyocytes in combination with a fluorescent calcium dye (FLIPR Calcium 6 Assay Kit, Molecular Devices) to develop a high-throughput drug screening assay on the FLIPR Tetra® (Molecular Devices) screening platform. After optimization of the assay for application with 384-well plates, we investigated the impact of different cardioactive compounds (e.g. hERG-channel blockers, calcium channel agonists/ antagonists and β-adrenergic agonists) on the calcium transients in Pluricyte® Cardiomyocytes. To validate the results obtained with the FLIPR Tetra® screening platform, we compared the calcium transient data to the field potential data obtained by medium-throughput multielectrode array (MEA) assays. The results show that Pluricyte® Cardiomyocytes can be efficiently used to detect positive and negative chronotropic and inotropic compound effects, as well as proarrhythmic effects of test compounds at a high throughput. The calcium transient data of the cells showed a profile that was in line with the compound effects observed in MEA assays, supporting the potential of these cells for high-throughput screenings. We conclude that Pluricyte® Cardiomyocytes in combination with calcium flux assays on a high-throughput drug screening system provide a highly relevant in vitro assay to study cardiac safety and efficacy of pharmaceuticals.

Funding Source: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 726513

F-3084

OPTIMIZATION OF ASSAYS FOR CHARACTERIZATION OF CARDIOMYOCYTES DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) may provide a personalized in-vitro platform for disease modeling, high-throughput drug screening and screening for patients with cardiovascular diseases. To achieve optimal applications of hiPSC-CMs, our study contains two parts. The first part is to optimize assays for characterizing the electrophysiological and structural phenotype of aligned tissue chips seeded with hiPSC-CMs. The second part is to assess gene expression during the differentiating process of hiPSC-CMs under different protocols, aiding further optimization of the differentiation protocol. In the first part, three intracellular calcium transient assays were optimized and compared firstly on hiPSC-CM monolayers. Compared to EarlyToxTM Cardiotoxicity Kit (1h incubation) our positive control, the Fluo-4 assay (concentration 1:1000, 1h-incubation) revealed fluorescence signals with a higher amplitude ($p=0.0044$). We also evaluated the Rhod-2 assay which could be combined with membrane potential (voltage) assay, but it was excluded due to obvious cell toxicity. Fluo-4 assay revealed a peak amplitude ($p < 0.05$) at 1hr post wash with maximal cell viability. Next we utilized

our tissue chips and detected the calcium signals using the Fluo-4 assay. We discovered a decrease in signal amplitude of 50% which was then improved by using Tyrode's solution to 150%. FluoVoltTM Membrane Potential assay on tissue chips was also improved (13 fold) by using Tyrode's solution as the loading buffer instead of hiPSC-CM culture media. To evaluate chip structure, we used multi label staining of cardiac markers, which revealed local positive staining of NKX2-5, a cardiac transcription factor in early development, and partially disorganized sarcomeres and cell-to-cell alignment. Gene expression analysis of differentiating hiPSC-CMs revealed that expression levels of 6 markers associated with cardiac development and maturation varied among differentiation protocols used and the expression pattern was consistent with native heart tissue development. In conclusion, we have optimized assays to assess structure and function of our aligned tissue chips as well as have preliminary gene expression data helping identify better protocol for hiPSC-CM differentiation.

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